The Crystal Structure of DJ-1, a Protein Related to Male Fertility and Parkinson’s Disease*

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DJ-1 is a multifunctional protein that plays essential roles in tissues with higher order biological functions such as the testis and brain. DJ-1 is related to male fertility, and its level in sperm decreases in response to exposure to sperm toxicants. DJ-1 has also been identified as a hydperoxide-responsive protein. Recently, a mutation of DJ-1 was found to be responsible for familial Parkinson’s disease. Here, we present the crystal structure of DJ-1 refined to 1.95 Å resolution. DJ-1 forms a dimer in the crystal, and the monomer takes a flavodoxin-like Rossmann-fold. DJ-1 is structurally most similar to the monomer subunit of protease I, the intracellular cysteine protease from Pyrococcus horikoshii, and belongs to the Class I glutamine amidotransferase-like superfamily. However, DJ-1 contains an additional α-helix at the C-terminal region, which blocks the putative catalytic site of DJ-1 and appears to regulate the enzymatic activity. DJ-1 may induce conformational changes to acquire catalytic activity in response to oxidative stress.

DJ-1 was initially identified as a novel oncogene product that transforms mouse NIH3T3 cells in cooperation with activated Ras. DJ-1 is an ~20-kDa protein comprising 189 amino acid residues ubiquitously expressed in various human tissues and with a particularly high level of expression in the testes (1). SP22 or CAPI, a rat homologue of human DJ-1, was subsequently identified as a key protein related to infertility in rats exposed to sperm toxicants such as ornidazole and epichlorohydrin where DJ-1/CAPI/SP22 levels in the sperm and epididymis decreased with increased rat infertility (2–4). With the exception of DJ-1, no other protein decreased in response to exposure to sperm toxicants, supporting the close relationship between DJ-1 function and male fertility. Recently, Klínefelter et al. (5) revealed that DJ-1/CAPI/SP22 was located on the equatorial segment of the matured sperm head and anti-SP22 Ig significantly inhibited the in vitro fertilization of hamster oocytes. Thus, DJ-1 may play a role in both zona penetration and membrane fusion steps of fertilization (5, 6).

PIASxα was isolated as a DJ-1-binding protein, which is specifically expressed in the testes and down-regulates the transcriptional activity of the androgen receptor. DJ-1 directly binds to the androgen receptor binding site of PIASxα and absorbs PIASxα from the androgen receptor-PIASxα complex. Thus, DJ-1 is considered to be a positive regulator of androgen receptor-dependent transcriptional activity (7).

Interestingly, a highly conserved amino acid residue Leu-166 in DJ-1 was recently reported to be replaced by Pro in patients with familial Parkinson’s disease, PARK7, and thus, this mutation was considered to be responsible for Parkinsonism (8). DJ-1 was also identified as a hydperoxide-responsive protein, which is converted into a pI variant in response to oxidative stress as with H2O2 or paraquat, resulting in the production of reactive oxygen species. Thus, DJ-1 functions as a sensor for oxidative stress (9, 10). Since oxidative stress is closely related to neurodegenerative diseases, there is a great demand for the clarification of the relationship between the DJ-1 mutation and pathogenesis of Parkinson’s disease (11).

Although DJ-1 is a small protein of ~20 kDa, it is related to cell transformation, male fertility, oxidative stress response, and Parkinson’s disease. However, the molecular mechanism by which DJ-1 exerts these multiple functions remains elusive. Here, we report the first x-ray crystal structure of DJ-1 to get an insight into its functional properties.

**MATERIALS AND METHODS**

*Data Collection—Protein expression, purification, and crystallization will be described elsewhere. All of the diffraction data were collected at 100 K on a RAXIS IV imaging plate detector (Rigaku) using CuKα radiation from a rotating anode x-ray generator. The data collection was performed at a total oscillation range of 142° with a step of 2° for each exposure time of 60 min. The camera distance was 130 mm. The crystal was found to diffract to a resolution of up to 1.95 Å and belong to space group P31, with unit-cell parameters of a = b = 75.04 and c = 74.88 Å. The crystal contains two molecules in an asymmetric unit and has a solvent content of 59%. Iridium and mercury derivatives were prepared by soaking the crystals in a reservoir solution containing heavy atom reagents at 293 K. Selenomethionine derivative was expressed in Escherichia coli B834(DE3) using an amino acid medium (12) containing selenomethionine instead of methionine. The diffraction data of those derivatives were collected in the same conditions as the native one with the exception that the oscillation range was 180°. All of the data were processed using DENZO and SCALEPACK programs (13). The results of the diffraction data are summarized in Table I.

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Structure Determination of DJ-1—The initial phasing was performed by multiple isomorphous replacement method using the crystals of the three derivatives. All of the programs used were attached to the CNS program suite (14). After scaling was applied to the data sets of the derivatives, the heavy atom parameters were refined and the multiple isomorphous replacement phases were calculated using the data between resolution of 50 and 2.8 Å. After density modification (15) was applied to the multiple isomorphous replacement map, an initial model consisting of one DJ-1 molecule was placed on the modified electron density map. The other monomer was then generated through non-crystallographic symmetry operations. Initial refinement was performed by the torsion angle molecular dynamic simulated annealing method and bulk-solvent correction against the maximum-likelihood amplitude target. For each cycle, the model was rebuilt manually using the molecular modeling program Turbo-Frodo (16). Throughout the initial refinement, non-crystallographic symmetry constraints were imposed on all of the residues. After the resolution was extended to 2.5 Å, the constraints were lifted and refinement was performed by energy minimization, individual isotropic B factor refinement, and bulk-solvent correction against the maximum-likelihood amplitude target.

RESULTS

Characterization of DJ-1—DJ-1 was cloned into a pGEX6P vector and expressed in E. coli BL21(DE3) as a fusion protein with glutathione S-transferase. The protein was excised by trypsin and purified by gel exclusion chromatography. Molecular weight analysis by matrix-assisted laser desorption ionization time-of-flight/mass spectrometry and N-terminal amino acid sequence analysis revealed that the purified protein was intact DJ-1 (1–189). The molecular weight in solution was estimated to be 44 kDa by gel exclusion chromatography, suggesting that DJ-1 exists as a dimer in aqueous solution.

Overall Structure of DJ-1—The crystal structure of DJ-1 was solved by multiple isomorphous replacement. The electron density map after density modification was of sufficient quality to allow tracing of most residues in the structure. The model was subsequently refined to 1.95 Å with R = 17.1% and Rfree = 19.4%, respectively. All of the data collection and phasing and refinement statistics are summarized in Table I.

The final model contains two DJ-1 molecules in an asymmetric unit that form a face-to-face dimer with a 2-fold axis (Fig. 1) and 323 water molecules. The C-terminal Asp-189 is missing because of structural disorder. The dimer formation in the crystal is consistent with the result of gel exclusion chromatography, supporting the notion that the dimer form in the crystal is not due to crystal packing but is physiologically relevant. The overall structure of the DJ-1 dimer is globular with dimensions of 56.1 × 49.5 × 59.6 Å.

Structure of the DJ-1 Monomer—The DJ-1 monomer takes a flavodoxin-like Rossmann-fold, which contains a parallel β-sheet arranged in the order of β2-β1-β4-β5-β7 as a core (17). The β-sheet is flanked by α-helices so that DJ-1 has a three-layered structure (Fig. 2a). In addition, there are several secondary structural elements associated with the core, β3, α3, α6, α7, β6, and α9. In particular, β6 forms an anti-parallel β-sheet with β7. The DJ-1 monomer contains seven β-strands and nine α-helices in total (Fig. 2, a and b). Structure-based sequence alignment was made using human, mouse, Xenopus, nematoda, and Drosophila DJ-1 and CAP1/SP22, a rat homologue of DJ-1. Most of the conserved residues are involved in the structural core.

Dimer Interface of DJ-1—The dimer interface and the opposite surface of DJ-1 are shown in an electrostatic surface potential presentation (Fig. 3, a and b). The total area of the buried surface is ~2,600 Å². The dimer interface comprises β3, α1, α8, and α9 (Fig. 1). It should be noted that the intermolecular β-sheet is formed among the Val-51, Ile-52, and Cys-53

| Data collection statistics | Native | Se-met | K4IrCl6 | CH3HgCl |
|----------------------------|--------|--------|--------|--------|
| Resolution (Å)             | 100-1.90 | 100-2.5 | 100-2.5 | 100-2.5 |
| Concentration (mm)         | 10     | 10     | 0.1    | 0.1    |
| Wavelength (Å)             | 1.5418 | 1.5418 | 1.5418 | 1.5418 |
| No. of reflections         | 145,149 | 85,565 | 87,596 | 87,348 |
| No. of unique reflections  | 34,488 | 15,043 | 15,328 | 15,348 |
| Completeness (%)           | 98.2   | 100    | 100    | 100    |
| Rmerge a                   | 0.073  | 0.054  | 0.074  | 0.143  |

Phasing statistics

| Resolution (Å)              | 50-2.8 | 50-4.0 | 50-2.8 | 50-2.8 |
| No. of sites                | 10     | 6      | 8      | 8      |
| Rmerge b                    | 0.152  | 0.215  | 0.212  |
| Weighted set                | 0.61   | 0.69   | 0.60   |
| Phase set                   | 0.60   | 0.84   | 0.59   |
| FOM c                       | 0.642  |

Refinement statistics

| Resolution (Å)              | 37.52-1.95 |
| No. of reflections           | 32,766    |
| Completeness (%)             | 95.3      |
| R-factor                     | 0.171     |
| Free R-factor                | 0.194     |
| No. of protein atoms         | 2,720     |
| No. of water molecules       | 323       |
| R.m.s.d.d                      | 0.005     |
| Bond length (Å)              | 1.338     |

Notes:

- Rmerge = Σ||F₁|−|F₂||/Σ|F₁|, where (j) is the average intensity of reflection j for its symmetry equivalents.
- Rmerge = Σ|F₁|−|F₂|/Σ|F₁|, where F₁ and F₂ are the derivative and native structure-factor amplitudes, respectively.
- Rmerge = Σ|F₁|−|F₂|/Σ|F₁|, where F₁ is the calculated heavy atom structure-factor amplitude.
- FOM, mean figure of merit.
- R.m.s.d., root mean square deviation.
The Crystal Structure of DJ-1

Structural Similarity of DJ-1 to Other Proteins—Comparison of the DJ-1 structure with the Protein Data Bank (18) data base using the DALI search engine (19) revealed that DJ-1 is structurally most similar to the monomer unit of protease I, an intracellular cysteine protease from Pyrococcus horikoshii with a Z score of 26.3 and a root mean square deviation of 1.6 Å for 166 residues (Fig. 4, a and b) (20). The DALI search also revealed that DJ-1 has similar topology to three proteins: the domain of catalase HPII from E. coli (17, 21); the subunit of anthranilate synthase TrpG from Sulfolobus solfataricus (22, 23); and the domain of GMP synthetase from E. coli (Table II) (24). All of the three proteins have flavodoxin-like Rossmann folds and belong to the Class I glutamine amidotransferase-like superfamily (GAT superfamily) involving thiJ domains (17).

With the exception of the domain of catalase HPII where the catalytic cysteine residue is replaced by glycine, all of the proteins belonging to the GAT superfamily have hydrolase activity and contain Cys-His or Cys-His-Asp/Glu as a catalytic group (17, 20–24). The catalytic cysteine residue is structurally well conserved in the GAT superfamily and is located on the short kinked loop connecting an α-helix and a β-strand characterized as the “nucleophile elbow” in α/β-hydrolases (25). As a result, the catalytic cysteine residue falls in an unfavorably allowed region in the Ramachandran plot (25). Actually, in DJ-1, the connecting loop between β5 and α5 was found to form the nucleophile elbow similar to protease I (Fig. 4, c and d). Cys-106 is located on the loop and has an unfavorable main chain conformation. His-126 is the putative catalytic residue located in close proximity to Cys-106. The residues around the putative catalytic site in DJ-1 are also well conserved in DJ-1 homologues (Fig. 3c, residues encircled with a dotted line). However, there are no acidic residues around His-126 in DJ-1 monomer, whereas in protease I, the neighboring molecule provides Glu-74 to form the catalytic triad (Fig. 4d). Thus, we located on each β3 strand. The distance between the sulfur atoms of Cys-53 is 3.1 Å, slightly far apart to form a disulfide bridge. The interaction between α-helices is mainly hydrophobic but there are several hydrogen bonds and ionic interactions. A number of hydrophobic interactions including the following residues, Met-17, Val-20, Ile-21, Val-23, Val-50, Ile-52, His-126, Phe-162, Pro-184, Leu-185, and Val-186, were observed where half of them (Met-17, Ile-21, His-126, Pro-127, Pro-158, and Phe-162) were completely conserved in human, mouse, rat, Xenopus, Drosophila, and nematoda DJ-1. In particular, Met-17 and Phe-162 are the core of the hydrophobic interactions and are essential for dimer formation. The conserved (red) and type-conserved (yellow) residues are mapped on the surface of DJ-1. Notably, the dimer interface consists of the conserved and the type-conserved residues (Fig. 3c, encircled with a solid line) in contrast to the opposite surface (Fig. 3d), suggesting that the dimer formation of DJ-1 is correlated with its biological functions.

The overall structure of the DJ-1 dimer. A stereo pair of a ribbon diagram of the DJ-1 dimer. The ribbons are colored blue for monomer A and green for monomer B. The four structure elements responsible for the dimer interface are labeled in red. β3 is formed by dimerization. This figure and Figs. 2a and 4 were prepared using MolScript (27) and Raster3D (28).

The structure of the DJ-1 monomer. a, a ribbon diagram of the DJ-1 monomer. The secondary structure elements are shown in blue for α-helices and in green for β-strands and are labeled in the figure. Positions of the N and C termini are indicated with arrows. b, topology diagrams of DJ-1 (color-coded as in a). The α-helices are represented by rectangles, and β-strands are represented by arrows.

Surface representations of the dimer interface and the opposite surface of DJ-1. The electrostatic surface potential of DJ-1 for the dimer interface (a) and the opposite surface (b) is shown. Red and blue represent negative and positive potentials, respectively. The surface model for the dimer interface (c) and the opposite surface of DJ-1 (d) in which the binding surface is encircled with a solid line are shown. The conserved and type conserved residues are shown in red and yellow, respectively. Compared with the opposite surface, the residues on the dimer interface are either conserved or type-conserved. Notably, the residues forming the putative catalytic site (encircled with a dotted line) are located close to the dimer interface and are highly conserved. Fig. 3 was prepared using GRASP (29).

Structural Similarity of DJ-1 to Other Proteins—Comparison of the DJ-1 structure with the Protein Data Bank (18) data base using the DALI search engine (19) revealed that DJ-1 is structurally most similar to the monomer unit of protease I, an intracellular cysteine protease from Pyrococcus horikoshii with a Z score of 26.3 and a root mean square deviation of 1.6 Å for 166 residues (Fig. 4, a and b) (20). The DALI search also revealed that DJ-1 has similar topology to three proteins: the domain of catalase HPII from E. coli (17, 21); the subunit of anthranilate synthase TrpG from Sulfolobus solfataricus (22, 23); and the domain of GMP synthetase from E. coli (Table II) (24). All of the three proteins have flavodoxin-like Rossmann folds and belong to the Class I glutamine amidotransferase-like superfamily (GAT superfamily) involving thiJ domains (17).
searched for the possibility to form the catalytic triad in the DJ-1 dimer but did not find any acidic residues. Structural comparison between DJ-1 and protease I revealed that DJ-1 contains an additional a9 at the C terminus, which distinguishes DJ-1 from the rest of the GAT superfamily proteins. a9 and the C-terminal region appear to block the catalytic site of the DJ-1 counterpart and are endowed with regulatory roles (Fig. 4c). His-126 is involved in the dimer formation through hydrogen bond interaction with Pro-184 and the hydrophobic interaction with Val-186 of the counterpart, which imposes an unfavorable orientation on the His-126 imidazole ring to form the catalytic dyad and inhibits substrate binding (Fig. 4c). Furthermore, a9 may prevent the possible formation of the catalytic triad with an acidic residue on the counterpart as in protease I (Fig. 4d). We propose that in the crystal form, DJ-1 does not have any catalytic activity due to blockage by a9 and the C-terminal region but may have a catalytic function after conformational change induced by specific signals or protease digestion. Although we tested the protease activity of DJ-1 using synthetic substrates, we observed only negligible protease activity in its intact form (data not shown). DJ-1 is localized on the equatorial segment of the sperm head where the sperm fuses with oocytes as the sperm matures. In addition, anti-SP22 Ig significantly inhibited in vitro fertilization of hamster oocytes (5). Taken together, these results led us to speculate that DJ-1 plays an essential role in zona penetration and in promoting the fusion steps of fertilization where the protease activity of DJ-1 may be tightly regulated.

**Structural Implication for Parkinson’s Disease**—A DJ-1 mutation at Leu-166 to Pro was recently found to be associated with PARK7, a monogenic form of human Parkinsonism. Leu-166 is located at the middle of a8, and the mutation appears to break the α-helix. Leu-166 forms a hydrophobic interaction with Val-181, Lys-182, and Leu-187 on a9 and the C-terminal tail (Fig. 4c) so that the proline mutation would disrupt the hydrophobic interaction between a8 and a9, destabilizing the dimer interface of DJ-1. Notably, DJ-1 expression is induced by oxidative stress as well as H2O2 or paraquat and is regarded as an oxidative stress-responsive protein (9, 10). Because reactive oxygen species produced in normal dopamine metabolism have been implicated in neuronal death, oxidative stress in the brain is closely related to the pathogenesis of Parkinson’s disease (11). In this context, it is reasonable to assume that DJ-1 functions as an antioxidant protein and any defects may be the cause of Parkinson’s disease.

**DISCUSSION**

DJ-1 is a multifunctional protein and plays essential roles in tissues with higher order biological functions such as the testes and brain. Anti-DJ-1 Ig inhibited the fusion of sperm with an oocyte. Moreover, DJ-1 levels in sperm decreased upon exposure to sperm toxicants and is thought to be responsible for male fertilization. Considering the structural similarity between DJ-1 and the GAT superfamily proteins, we speculate that DJ-1 has protease activity that is inactive in the dimer structure but becomes active by conformation change or protease digestion at the C-terminal region.

DJ-1 was also identified as an oxidative stress responsive protein and was found to be associated with Parkinsonism, a neurodegenerative disease, supporting the notion that DJ-1 is responsible for the quality control of proteins under oxidative stress. Upon oxidative stress, pI of DJ-1 was reported to change from 6.2 to 5.8, suggesting that DJ-1 might adsorb the reactive oxygen species and is modified to acquire a slightly lower pI. Oxidative conversion of sulfhydryl group(s) at a cysteine residue(s) to a cysteine sulfenic acid (Cys-SO2H) is the most plausible candidate responsible for the pI shift of hydroperoxide-responsive proteins (9). Mutation of Cys-53 to Ala actually

![Image of structures](http://www.jbc.org/)

**Fig. 4. Comparison of the monomer structures and the putative catalytic sites between DJ-1 and protease I.** Ribbon diagrams of the monomer subunits of DJ-1 (a) and protease I (b). Secondary structure is color-coded as in Fig. 2a. c, the region around the putative active site of DJ-1 including β5, α5, and the nucleophile elbow in monomer A (in blue) and α8 and α9 in monomer B (in green). The residues Cys-106, His-126, and Val-128 in monomer A and Leu-166, Val-181, Lys-182, Pro-184 (a backbone oxygen), Val-186, and Leu-187 in monomer B are shown as ball-and-stick models. The His-126 imidazole ring forms hydrogen bonds with the main-chain carbonyl group of Pro-184 (monomer B) and the main-chain amide group of Val-128 (monomer A) as shown by the dotted lines. Thus, the His-126 imidazole ring does not take a preferable orientation for protease activity. Leu-166, mutated to proline in PARK7 patients, is shown in red. d, the region around the active site of protease I including the nucleophile elbow in which the catalytic residue Cys-100 is located. Protease I forms a hexamer, and the catalytic triad is formed in the dimer interface (monomer A is in blue, and monomer B is in green).

**TABLE II**

| Protein                          | Ca * | Z-score | R.m.s.d. | Identity | PDB code |
|----------------------------------|------|---------|----------|----------|----------|
| Protease I                       | 166  | 26.3    | 1.6      | 22       | 1g2i-A   |
| Catalase HPII                    | 156  | 16.5    | 2.0      | 11       | 1c69-A   |
| Anthranilate synthase (TrpG-subunit) | 122  | 7.9     | 3.2      | 12       | 1qdl-B   |
| GMP synthetase                   | 122  | 7.2     | 3.1      | 10       | 1gpm-A   |

* The structures were compared to that of DJ-1 using the DALI program.

* Number of C atoms superimposed between the two structures.

* R.m.s.d., root mean square deviation of superimposed C atoms.

* The sequence identity is given between the number of Ca atoms superimposed.
abolished the formation of the pI variant, confirming that Cys-53 is responsible for oxidative stimuli. Since Cys-53 is located on β3 of the dimer interface, the conversion of Cys to Cys-SO₂Hi n in β3 may destabilize the dimer interface and the DJ-1-specific C-terminal region may be displaced, thus removing the inhibition of protease activity. Proline mutation may abolish the conformation change required for protease activity.

The crystal structure of a heat shock protein, E. coli Hsp31, was recently reported (26). Interestingly, its structure is quite similar to that of DJ-1, although an additional domain donates the acidic residues, resulting in the formation of the catalytic triad. However, the catalytic site is completely covered by the inserted domain, which develops a fused hydrophobic surface on the Hsp31 dimer. Thus, Hsp31 appears to sense oxidative stress and gains the protease activity to digest oxidative damaged proteins.

Although further studies are required to elucidate the relationship of DJ-1 with male fertility, oxidative stress, and Parkinson’s disease, the crystal structure of DJ-1 has shed light on the structure-function relationship of DJ-1.

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