Crystal Structure of Human DJ-1, a Protein Associated with Early Onset Parkinson’s Disease*

Xiao Tao and Liang Tong‡
From the Department of Biological Sciences, Columbia University, New York, New York 10027

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We report the crystal structure at 1.8-Å resolution of human DJ-1, which has been linked to early onset Parkinson’s disease. The monomer of DJ-1 contains the α/β-fold that is conserved among members of the DJ-1/ThiJ/PfpI superfamily. However, the structure also contains an extra helix at the C terminus, which mediates a novel mode of dimerization for the DJ-1 proteins. A putative active site has been identified near the dimer interface, and the residues Cys-106, His-126, and Glu-18 may play important roles in the catalysis by this protein. Studies with the disease-causing L166P mutant suggest that the mutation has disrupted the C-terminal region and the dimerization of the protein. The DJ-1 proteins may function only as dimers. The Lys to Arg mutation at residue 130, the site of sumoylation of DJ-1, has minimal impact on the structure of the protein.

Parkinson’s disease (PD)† is a common, progressive neurodegenerative disorder affecting roughly 1% of the population at the age of 65 (1). Clinically, PD generally presents with bradykinesia, resting tremor, muscular rigidity, and postural instability. PD is a heterogeneous disease, and the majority of the cases appear to have sporadic origins. At the same time, the disorder can also be associated with specific genetic defects, especially for cases of familial PD (2–4). Ten different genetic loci have been linked with familial PD (2–4). One genetic disruption is a single point mutation, giving rise to the L166P protein. The other mutations involve disruptions of transcription; DJBP, DJ-1-binding protein; GAT, glutamine amidotransferase; r.m.s., root mean square; HPII, hydroperoxidase II.

The exact biological function of the DJ-1 protein is currently unknown. It may play a role in the oxidative stress response, and this function could be important in preventing the onset of PD (8). Both α-synuclein and parkin participate in oxidative stress responses as well (9, 10). On the other hand, DJ-1 may also be associated with several other biological processes. It was first identified as an oncogene, because it can transform NIH3T3 cells in cooperation with the ras oncogene (11). The DJ-1 protein is also involved in the fertilization process in rat and mouse (12–14). A significant reduction in the amount of this protein on the surface of sperm makes them unable to fertilize eggs (15). This finding suggests that the protein may be secreted under some circumstances, which has also been observed in breast cancers (16). Finally, DJ-1 was identified as the regulatory subunit of a 400-kDa RNA-binding protein complex and its presence inhibits the binding of RNA by the complex (17).

The DJ-1 protein is a positive regulator of the androgen receptor by sequestering its negative regulators PIASxα (protein inhibitor of activated STAT) or DJBP (DJ-1-binding protein) (18, 19). The activation of the androgen receptor might be related to the effects of this protein on fertility. PIAS proteins are SUMO-1 (small ubiquitin-like modifier-1) ligases and control their target proteins by sumoylation (20). It has been reported that PIASxα can sumoylate DJ-1 on the Lys-130 residue (18). DJBP is almost exclusively expressed in the testis in humans. It negatively modulates the androgen receptor by recruiting histone deacetylases. DJ-1 can inhibit this recruitment, thereby leading to the activation of the androgen receptor (19).

The human DJ-1 protein contains 189 amino acid residues. It belongs to the DJ-1/ThiJ/PfpI superfamily of proteins, which are conserved in many different organisms (Fig. 1). The function of ThiJ is currently unknown, although it might be related to the biosynthesis of thiamin (21, 22). PfpI is an intracellular protease that was first identified from the archaeon Pyrococcus furiosus (23), and it is present in most bacteria and archaea. The crystal structure of the closely related intracellular protease PH1704 from Pyrococcus horikoshii revealed the presence of a Cys-100/His-101/Glu-74 catalytic triad (primed residue indicate a different monomer) with the active site at the interface of neighboring monomers in a hexameric oligomer (24). A Cys-185/His-186/Asp-214 catalytic triad was found in a structural homolog of this protease, the heat shock protein Hsp31 from E. coli (25). The amino acid sequence of this chaperone, however, shares only a 19% identity with that of PH1704. The DJ-1/ThiJ/PfpI proteins also share limited sequence and structural homology to those of the type I glutamine amidotransferase (GAT) domains, which contain a Cys-His-GluAsp catalytic triad (26).

Although the Cys residue in the active site of the PfpI proteases is conserved among all of the members of the DJ-1/ThiJ/PfpI superfamily, the His and Asp/Glu residues are not con-
served in DJ-1 and its close homologs (Fig. 1). To determine the composition of this putative active site in DJ-1 and to obtain a better understanding for the possible molecular function of this protein, we have determined its crystal structure at a 1.8-Å resolution. We have also characterized the disease-causing L166P mutant in solution, which suggests that the DJ-1 protein may function only as a dimer.

MATERIALS AND METHODS

**Protein Expression and Purification**—The gene for human DJ-1 was subcloned into the pET24d vector and overexpressed in *E. coli* at 20°C. The recombinant protein contains a hexa-histidine tag at the C terminus. After cell lysis, the soluble protein was purified by nickel-agarose affinity, anion exchange, and gel-filtration chromatography. The protein was concentrated to 30 mg/ml in a buffer containing 20 mM Tris (pH 8.0), 150 mM NaCl, 3 mM DTT, and 5% (v/v) glycerol and stored at −80°C. The recombinant protein was at a concentration of 15 mg/ml (w/v) polyethylene glycol 3350. The protein was concentrated pre-mixed with 100 mM Bis-Tris (pH 8.0), 25% (w/v) PEG3350, and 1.3 Å to 2.2 Å. The locations of four Se atoms were determined with the program SnB (29). Reflection phases to 1.8-Å resolution were calculated based on the single wavelength anomalous diffraction data set to 1.7-Å resolution. The full atomic model was built into the electron density with the program O (31). The structures of the monoclinic crystal form and the K130R mutant were determined by the molecular replacement method with the program COMO (32). The structure refinement was carried out with the program CNS (33) with the experimental data with a R-free value of 14.0%–15.0% for the wild-type protein and a R-free value of 18.0%–19.0% for the K130R mutant.

**RESULTS AND DISCUSSION**

**The Structure Determination**—The crystal structure of wild-type human DJ-1 protein in a trigonal crystal form has been determined at 1.8-Å resolution by the selenomethionyl single wavelength anomalous diffraction method (34). The positions of the Se sites were determined by direct methods (29), which also automatically located 80% of the residues in the molecule. The full atomic model contains residues 2–188 of DJ-1 together with 199 water molecules. The last residue of the protein and the C-terminal hexa-histidine tag (LEHHHHHH) are not visible in the electron density map. The atomic model has excellent agreement with the experimental data with a R-factor of 19.0% (Table I). The r.m.s. deviation from ideal values in bond lengths and bond angles are 0.004 Å and 1.3°, respectively. All of the residues with the exception of Cys-106 are located in favored regions of the Ramachandran plot.

**FIG. 1.** Amino acid sequences of the DJ-1/ThiJ/PfpI superfamily. The sequences of representative members of the three subfamilies are shown. A dot represents a deletion. The secondary structure (S.S.) elements in the DJ-1 structure are shown. Residues in the core of the DJ-1 monomer are colored in green, those in the dimer interface are in magenta, and those in the putative active site are in red.
the Ramachandran plot (data not shown). The Cys-106 residue has a strained main chain conformation (see below).

We have also determined the crystal structure at 2.2-Å resolution of the wild-type protein in a monoclinic crystal form (Table I) by the molecular replacement method (32, 35). This crystal contains four dimers of DJ-1 in the asymmetric unit, allowing us to assess the conformational flexibility of the monomer and dimer of DJ-1.

The structure of the K130R mutant of human DJ-1 has been determined at 1.7-Å resolution by the molecular replacement method (Table I). This crystal form is not isomorphous to either of the native crystals. The structure shows that the K130R side chains in both monomers are involved in crystal-packing interactions with neighboring dimers in the crystal, and these interactions are different from those for the Lys-130 side chain in the wild-type crystals.

**Structure of the DJ-1 Monomer**—The structure of the monomer of DJ-1 has the α/β-fold with 11 β-strands (β1–β11) and 8 α-helices (αA–αH) (Fig. 2A). The central β-sheet of the structure contains seven strands. The six parallel strands of this sheet are arranged similar to those in the Rossmann-fold with the distinction that the third strand is very short in the DJ-1 structure (β3 with only two residues) (Figs. 1 and 2A). Outside the central β-sheet, strands β3 and β4 form a β-hairpin structure and are involved in the dimerization of DJ-1 (see below). Strands β5 and β7 together with helix αF form a β-α-β motif, which contributes the conserved His-126 residue to the putative active site of DJ-1 (Fig. 2A).

Most of the helices flank the two faces of the β-sheet (Fig. 2B). The only exception is helix αH at the extreme C terminus of DJ-1. It projects away from the rest of the protein and only contacts helices αA and αG in the monomer (Fig. 2B). Residues 181–187 in helix αH are conserved to be hydrophobic amino acids among the DJ-1 and ThiJ proteins (Fig. 1). These residues mediate the interactions with helices αA and αG as well as the dimerization of DJ-1 (see below).

**The Dimer of DJ-1**—Gel-filtration and light-scattering studies showed that wild-type DJ-1 protein exists as dimers in solution. In the trigonal crystal of the wild-type protein, there are four dimers in the asymmetric unit. The other monomer of the dimer is related by the crystallographic 2-fold symmetry axis (Fig. 3A). The αA helices of the two monomers come into close contact in the core of the dimer (Fig. 3B). At one edge of the interface, the β3-β4 hairpin is situated right next to the dimer 2-fold axis, such that a four-stranded anti-parallel β-sheet is formed across the dimer (Fig. 3A). At the other edge, the β11-αG loop interacts with residues near the C terminus of the other monomer, including those in helix αH (Fig. 3B).

These interactions may also be important to stabilize the conformation of the αH helix at the C terminus of DJ-1.

In the monoclinic crystal form of the wild-type protein, there are four dimers in the asymmetric unit. Structural comparisons among these dimers and the dimer in the trigonal crystal form show that the organizations of the dimers are essentially the same. The r.m.s. distance between equivalent Ca atoms of any pair of these dimers is approximately 0.35 Å. The αH helix shows conformational variability among the four dimers in the monoclinic crystal, and this helix also has weak electron density in the trigonal crystal. The organization of the dimer is also conserved in that of the K130R mutant with a r.m.s. distance of 0.3 Å for 374 pairs of equivalent Ca atoms of the wild-type and mutant dimers.

The DJ-1 dimer has an extensive interface between the two monomers. A total of 1200 Å² of the surface area of each monomer is buried at this interface, involving mostly residues that are conserved among DJ-1 proteins (Fig. 1). Our structural and solution observations therefore suggest that this dimer is likely to be a stable and conserved oligomerization state for these proteins.

Interestingly, a deletion mutant of DJ-1 removing residues 178 to the C terminus (Fig. 1) is still mostly dimeric in solution (data not shown), suggesting that either the αH helix has a minor role in the dimerization of DJ-1 or the C-terminal hexahistidine tag (LEHHHHHH) is partially rescuing the dimer formation. On the other hand, the deletion mutant lacking residues 173 to the C terminus forms large aggregates of various sizes (molecular masses of 200–400 kDa) with little dimeric species in solution.

**Structural Homologs of DJ-1**—The structure of DJ-1 bears the strongest similarity to that of PH1704 from *P. horikoshii* (24), a member of the Pfpl family, and the two proteins share 22% amino acid sequence identity. The r.m.s. distance is 1.1 Å for 137 equivalent Ca atoms between DJ-1 and PH1704. Most of the secondary structure elements are superimposable between the two proteins (Fig. 2C). However, the αH helix at the C terminus of DJ-1 does not have a structural equivalent in PH1704. Sequence comparisons suggest that all of the Pfpl family members lack this helix, because they are approximately 15 residues shorter than DJ-1 at the C terminus (Fig. 1).

The quaternary structure of PH1704 and DJ-1 are entirely different, despite the homology in the sequence and structure of their monomers. In contrast to the dimeric association of DJ-1, a hexameric structure roughly obeying 32 symmetry was observed in the crystal of PH1704 (24). This hexamer can be considered as a trimer of dimers, and the interface of the dimer is mediated by the αA and αG helices. However, such a mode of dimerization is not possible with DJ-1 as it is blocked by the

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**Table I**

Summary of crystallographic information

| Protein       | Wild-type | Wild-type | K130R mutant |
|---------------|-----------|-----------|--------------|
| Space group   | P2₁2₁    | P2₁2₁     | P2₁         |
| Cell parameters (a, b, c, β) | 75.66, 75.66, 75.49 | 71.26, 83.66, 114.16, 100.56 | 77.71, 78.17, 63.82, 108.88 |
| Maximum resolution (Å) | 1.8 | 2.2 | 1.7 |
| Number of observations | 237,493 | 211,982 | 112,333 |
| Rwp (%)      | 4.9 (20.8) | 9.4 (23.1) | 6.5 (26.4) |
| Resolution range for refinement | 20–1.8 | 20–2.2 | 20–1.7 |
| Number of reflections | 41,325 | 61,031 | 36,794 |
| Completeness (%) | 92 (82) | 91 (83) | 93 (80) |
| ρ-factor (%) | 19.0 (21.9) | 18.0 (19.3) | 19.1 (21.1) |
| Free ρ-factor (%) | 21.5 (23.3) | 24.3 (27.5) | 23.5 (24.9) |
| R.m.s. deviation in bond lengths (Å) | 0.004 | 0.005 | 0.005 |
| R.m.s. deviation in bond angles (%) | 1.3 | 1.3 | 1.3 |

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* a Rwp = Σ|Iobs|−|Icalc|/ΣIobs|Icalc|.  
* b R = Σ||Fobs|−|Fcalc||/Σ|Fobs|Fcalc|.

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X. Tao and L. Tong, unpublished data.
Therefore, the DJ-1 dimer is formed by a different arrangement of the α and β helices of the two monomers and the αH helices also contribute to the dimerization interface (Fig. 3A). In addition, the contribution of the β3–β4 hairpin to the dimer interface is unique to DJ-1 (Fig. 3A). The dimerization of Hsp31, a structural homolog of PH1704 and DJ-1, is mediated by segments outside the Pfpl/DJ-1-fold (25).

Searches against the Protein Data Bank with the program Dali (36) showed that DJ-1 also shares significant structural similarity with the C-terminal domain in the catalase hydroperoxidase II (HPII) (37) as has been suggested earlier (26). The r.m.s. distance for 136 equivalent Ca atoms between DJ-1 and this C-terminal domain (residues 600–753) is 2.0 Å. The amino acid identity for these residues is however only 12%.

This domain in HPII lacks the β8–β10 insertion (Fig. 2D), which accounts for its smaller size. At the C terminus, HPII also has a helix but it is running in an opposite direction from the αH helix in DJ-1. The exact function of this C-terminal domain in HPII is not fully understood. It mediates the dimer interface in the tetramer of the enzyme and helps to form the substrate channel leading to the active site (37). This domain may also facilitate the folding of the full-length enzyme, as deletion mutants lacking this domain cannot accumulate in the cell. The dimerization of this domain in HPII uses the unique αH helix at the C terminus and is different from that in DJ-1.

Structural homology between the DJ-1/ThiJ/Pfpl superfamily and the GAT domains has also been suggested earlier (26). However, the actual similarity is rather limited. The GAT domains lack the β3–β4 hairpin structure in DJ-1 but contain
significantly more elaborate insertions between $\alpha E$ and $\beta 10$. Moreover, the binding site for glutamine in GAT is blocked by the $\beta 6-\alpha C$ loop in the DJ-1 structure. Therefore, it is unlikely that DJ-1 can have GAT function.

**The Putative Catalytic Residues of DJ-1**—The exact biochemical function of the DJ-1 protein is currently not known. However, structural and sequence comparisons with homologous proteins can provide valuable hints as to the possible roles of this protein in oxidative stress response, androgen receptor regulation, and other processes. A cysteine residue is conserved among all of the members of the DJ-1/ThiJ/PpI superfamily, and the residue has been proposed as the catalytic nucleophile for the PpI family of intracellular proteases (24). This residue is equivalent to Cys-106 in human DJ-1 (Fig. 1) and is located just after strand $\beta 7$ in the structure (Fig. 2A). The main chain of this residue assumes a strained conformation with $\phi$ and $\psi$ torsion angles of 66 and $-114^\circ$, respectively, which is also observed in the structures of PH1704 and Hsp31 (24, 25). The structural conservation of this Cys residue in DJ-1 suggests that it may also have a catalytic role in the biochemical function of this protein, identifying this region of the structure as the putative active site of DJ-1.

In both the PpI proteases and the Hsp31 chaperone, the Cys residue is part of a Cys-His-Asp/Glu catalytic triad (24, 25). The second member His residue in the triad follows immediately after the Cys residue in the primary sequence of these proteins.

**Fig. 3. Structure of the DJ-1 dimer.** A, schematic drawing in stereo of the dimer of DJ-1. One monomer is colored in cyan, whereas the other is in green. Secondary structure elements in the dimer interface are labeled. The side chains of residues Cys-106, His-126, and Glu-18 in the putative active site of the protein are also shown. B, structure of DJ-1 dimer in a different view after 90° rotation around the vertical axis. This figure was produced with Ribbons (39).

**Fig. 4. The putative active site of DJ-1.** A, schematic drawing of the structure near the Cys-106 residue of DJ-1. The two monomers are colored in green and cyan, respectively. B, molecular surface of the DJ-1 dimer near the putative active site. Panel A was produced with Ribbons (39), and panel B was produced with Grasp (40).
In DJ-1, however, the residue immediately after Cys-106 is Ala (Fig. 1), signifying that there are differences between DJ-1 and the PfpI proteases in this active site. Our structures show that His-126, from the \( \beta \)-H9251F loop and conserved among all of the DJ-1 proteins (Fig. 1), is placed near the Cys-106 side chain in the putative active site of DJ-1 (Fig. 4A). The His-126 residue could be the second member of the catalytic machinery for DJ-1. The distance between the side chains of Cys-106 and His-126 is 4.2 Å in the current structure (Fig. 4A) similar to the distance of approximately 5 Å that is found in the active site of caspases (38).

Simultaneous mutation of three residues of DJ-1, S57R, E96G, and H126Y can disrupt the interactions with PIASx (18), providing support for the functional importance of the His-126 residue. However, all of the three residues are strictly conserved among the DJ-1 proteins (Fig. 1) and both Ser-57 and Glu-96 appear to have important structural roles as well. Therefore, it is not clear which of the three mutations is responsible for the loss of activity. The three residues are separated by ~25 Å from each other in the structure, so it is unlikely that they can all mediate the interactions with PIASx at the same time.

In PH1704, the third member Glu residue of the catalytic triad comes from another monomer in the enzyme oligomer (Figs. 1 and 2C). In DJ-1, however, the residue immediately after Cys-106 is Ala (Fig. 1), signifying that there are differences between DJ-1 and the PfpI proteases in this active site. Our structures show that His-126, from the \( \beta \)-H9251F loop and conserved among all of the DJ-1 proteins (Fig. 1), is placed near the Cys-106 side chain in the putative active site of DJ-1 (Fig. 4A). The His-126 residue could be the second member of the catalytic machinery for DJ-1. The distance between the side chains of Cys-106 and His-126 is 4.2 Å in the current structure (Fig. 4A) similar to the distance of approximately 5 Å that is found in the active site of caspases (38).

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In PH1704, the third member Glu residue of the catalytic triad comes from another monomer in the enzyme oligomer (Fig. 2C) (24), whereas in Hsp-31, the third member Asp residue comes from an inserted segment to the DJ-1/ThiJ/PfpI-fold (25). In our structures of DJ-1, no acidic residues are positioned near the His-126 side chain. The active site of the DJ-1 family members may contain only a Cys-His diad. With a cysteine residue as the nucleophile, it is known that a Cys-His diad is fully functional, for example, in the caspases.

On the other side of the Cys-106 residue, the side chain of Glu-18 is within 3.2 Å of the S\( _7 \) atom in the structure of DJ-1 (Fig. 4A). This Glu residue is strictly conserved among all of the DJ-1/ThiJ/PfpI proteins (Fig. 1), and it is also present in Hsp31. Moreover, the side chain of this residue is completely buried in the structure. Therefore, it is likely that this residue also has an important role in the biochemical functions of these proteins.

The Putative Active Site of DJ-1—The putative active site of DJ-1 is located near the interface of the dimer with contributions from residues in helices \( \alpha \)-A and \( \alpha \)-H of the other monomer (Fig. 4A). The two monomers of the dimer are arranged in a head-to-tail fashion, such that the two active sites are on opposite sides of the dimer (Fig. 3A). The Cys-106 residue lies in the center of a prominent surface depression in the structure (Fig. 4B). Residues in this active site are generally highly conserved including the \( ^{74} \)GGNLGA\(^{79} \) motif (\( \beta \)-\( \alpha \)-C loop) and
the 157GPG159 motif (β8-αF loop) (Fig. 4A) that are conserved among the DJ-1 and ThiJ proteins (Fig. 1). The αA helix plays an especially important role in this active site. The Glu-18 residue is located near the N terminus of this helix, whereas Asp-24 and Arg-28 near its C terminus contribute to the other active site of the dimer (Fig. 4A).

Near Cys-106, there is a cluster of three acidic residues (Glu-15, Glu-16, and Asp-24') and two basic residues (Arg-48 and Arg-28') (Fig. 4A). These residues are highly conserved among all of the members of the DJ-I/ThiJ/PfpI superfamily (Fig. 1). The side chain of Glu-16 may stabilize the conformation of the β3-β4 hairpin by hydrogen bonding to residues 47 and 48 at its tip (Fig. 4A). Glu-15 is the N-terminal cap of helix αA and is hydrogen-bonded to the main chain amide of residue 17. In addition, this residue interacts with Asp-24' and Arg-28', helping the formation of the dimer. It is likely that Arg-48 and Arg-28' are involved in the binding of the substrate of this protein (Fig. 4A).

The Structure of the K130R Mutant, Site of Sumoylation—It has been reported that the Lys-130 residue can become sumoylated and that this modification is important for DJ-1 to achieve full activity in cell transformation (18). In the crystal structure, the Lys-130 residue is located at the beginning of helix αF, −10 Å from the His-126 residue, and its side chain is completely exposed to the solvent (Fig. 5). Our structure of the K130R mutant of DJ-1 showed that the mutation has little impact on the structure of the protein itself. The Arg-130 side chains in the two monomers assume different conformations (Fig. 5), but this is probably due to the fact that the two Arg residues are involved in different crystal-packing contacts. The structural observation is consistent with the fact that the K130R mutation does not disrupt the interaction with the PIASx family protein (18).

Studies on the Disease-causing L166P Mutant—The L166P mutation of human DJ-1 was first found in an Italian PD patient (8). This residue is strictly conserved among the DJ-1 proteins (8) as well as the ThiJ proteins, although it is not conserved among the PfpI proteases (Fig. 1). Based on sequence homology between DJ-1 and the PH1704 protease, it was suggested that the Leu-166 residue is located in a helix and the L166P mutation may disrupt the formation of this helix (8).

Our structural analyses confirm that Leu-166 is located in the center of helix αG (Fig. 2B). Moreover, our structures show that the side chain of this residue has a major role in forming the hydrophobic core between the extra αH helix at the C terminus and the αA and αG helices (Fig. 6). A total of 78 Å² of the surface area of Leu-166 is buried at this interface. Therefore, the L166P mutation may have two effects on the structure of DJ-1 destabilization/unfolding of the αG helix and the disruption of the hydrophobic core among helices αA, αG, and αH.

To further characterize the structure of the L166P mutant, we have expressed it in E. coli and purified the soluble protein by nickel affinity, anion exchange, and gel filtration chromatography. Efforts at producing crystals of this mutant have so far been unsuccessful. Light-scattering studies with the purified protein show that the mutant is monomeric in solution. This is consistent with the structural analysis that the L166P mutation may lead to the unfolding of the C-terminal segment of DJ-1, which will also disrupt the formation of the dimer. These results suggest that the DJ-1 protein may only function as a dimer.

Functional Implications—Based on overall sequence homology, the DJ-1, ThiJ, and PfpI proteins have been grouped together into a superfamily. With the structural information on DJ-1 and PfpI (PH1704), it is clear that there are significant structural and sequence differences among the members of the superfamily. DJ-1 and ThiJ proteins may be more closely related to each other, whereas the PfpI proteins are more distant. Examination of the amino acid sequence conservation suggests that ThiJ proteins might also have the αH helix at the C terminus (Fig. 1) similar to DJ-1, whereas the PfpI proteins do not. The conservation of the Leu-166 residue in the DJ-1 and ThiJ proteins is probably related to the presence of this extra helix, because it is a crucial component of the hydrophobic core between this helix and the rest of the protein (Fig. 6). It is also possible that the ThiJ proteins will form the same dimer as DJ-1, in contrast to PH1704. The disease-causing effects of the L166P mutation in DJ-1 underscores the functional importance of the structural integrity of the C-terminal segment and indicates that these proteins may function only as dimers.

Another difference among members of this superfamily is that the active site His residue immediately follows the catalytic Cys nucleophile in the primary sequence only in the PfpI proteins. Although DJ-1 and ThiJ are similar in maintaining an Ala residue at this position in the primary sequence, the His-126 residue identified from our structural studies is conserved only in DJ-1. Based on the structural information and the sequence alignment, it is unlikely that the ThiJ proteins contain a His residue in the active site. However, the Glu-18 residue is still conserved in ThiJ.

DJ-1 has been associated with many different biochemical and biological functions. Our structural analysis suggests that DJ-1 may possess an active site and could also be a protease/hydrolase similar to the PfpI proteases. The relative positioning of the Cys-106 and His-126 residues are reminiscent of the active site of caspases. In addition, Arg-48 and Arg-28' residues are approximately 8 Å away from the Cys-106 residue in DJ-1, similar to the distances for the two Arg residues that recognize the P1 Asp residue in the substrate of caspases. It would be interesting to determine whether DJ-1 can recognize Asp residues in this putative active site.

The DJ-1 protein can also mediate interactions with other proteins such as PIASxα, DJBP, and the RNA-binding protein complex. It is not known whether the putative catalytic activity of DJ-1 is required for these interactions or whether a different surface patch can mediate these interactions. The structural conservation between DJ-1 and a domain that is crucial for the function of HP1 and other catalases offers the tantalizing possibility that DJ-1 may also regulate the dismutation of peroxides. This could be the link between DJ-1 and the oxidative stress response.

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REFERENCES
1. Dawson, T. M., and Dawson, V. L. (2003) J. Clin. Invest. 111, 145–151
2. Gasner, T. (2001) J. Neurosci. 21, 833–840
3. Giasson, B. I., and Lee, V. M.-Y. (2001) Neuron 31, 885–888
4. Cookson, M. R. (2003) Nature 427, 7–10
5. Polymeropoulos, M. H., Lavedan, C., Leroy, E., Ide, S. E., Dehejaia, A., Dutra, A., Pike, B., Root, H., Rubenstien, J., Boyer, R., Sternson, E. S., Chandrasekharappa, S., Alhanyassadou, A., Papapetropoulos, T., Johnson, W. G., Lazzarini, A. M., Duvoisin, R. C., di Iorio, G., Gelbe, L. I., and Nussbaum, R. L. (1997) Science 276, 2045–2047
6. Kitada, T., Asakawa, S., Hattori, N., Matsunime, H., Yasumura, Y., Minoshima, S., Yokochi, M., Mizonou, Y., and Shimizu, N. (1998) Nature 392, 605–608
7. Liu, Y., Fallon, L., Lashuel, H. A., Liu, Z., and Lansbury, P. T., Jr. (2002) Cell 111, 209–216
8. Bonifati, V., Rizzu, P., van Baren, M. J., Schaap, O., Breedveld, G. J., Krüger, E., Dekker, M. C. J., Squitteri, F., Ibanez, P., Jousse, M., van Dongen, J. W., Varesco, L., van Swieten, J. C., Bruce, A., Meoo, G., van Diijn, C. M., Oosta, B. A., and Heutink, P. (2003) Science 299, 256–259
9. Hashimoto, M., Hsu, L. J., Rockenstein, E., Takanouchi, T., Mallory, M., and Masliah, E. (2002) J. Biol. Chem. 277, 11465–11472
10. Hyan, D.-H., Lee, M., Hattori, N., Kubo, S.-I., Mizuno, Y., Halliwell, B., and Jenner, P. (2002) J. Biol. Chem. 277, 28572–28577
11. Nagakubo, D., Taira, T., Kitaura, H., Ikeda, M., Tamai, K., Ighuchi-Ariga, S., and Oostra, B. A., and Heutink, P. (2003) Science 299, 256–259
12. Wagenfeld, A., Gommoll, J., and Cooper, T. G. (1998) Biochem. Biophys. Res. Commun. 251, 545–549
13. Welch, J. E., Barber, R. R., Roberts, N. L., Suarez, J. D., and Klinefelter, G. R. (1998) J. Androl. 19, 385–393
14. Okada, M., Matsumoto, K.-I., Niki, T., Taira, T., Iguchi-Ariga, S. M. M., and Ariga, H. (2002) Biol. Pharm. Bull. 25, 853–856
15. Wagenfeld, A., Yeung, C.-H., Shivaji, S., Sundareswaran, V. R., Ariga, H., and Cooper, T. G. (2000) J. Androl. 21, 954–963
16. Le Naour, F., Misek, D. E., Krause, M. C., Deneux, L., Giordano, T. J., Scholl, S., and Hanash, S. M. (2001) Clin. Cancer Res. 7, 3328–3335
17. Hod, Y., Pentyala, S. N., Whyard, T. C., and El-Maghrabi, M. R. (1999) J. Cell. Biochem. 72, 435–444
18. Takahashi, K., Taira, T., Niki, T., Seino, C., Iguchi-Ariga, S. M. M., and Ariga, H. (2001) J. Biol. Chem. 276, 37556–37563
19. Niki, T., Takahashi-Niki, K., Taira, T., Iguchi-Ariga, S. M. M., and Ariga, H. (2003) Mol. Cancer Res. 1, 247–251
20. Kotaja, N., Karvonen, U., Janne, O. A., and Palvimo, J. J. (2002) Mol. Cell. Biol. 22, 5222–5234
21. Mizote, T., Tsuda, M., Smith, D. D. S., Nakayama, H., and Nakazawa, T. (1999) Microbiology 145, 495–501
22. Taylor, S. V., Kelleher, N. L., Kinland, C., Chiu, H.-H., Costello, C. A., Backstrom, A. D., McLafferty, F. W., and Begley, T. P. (1998) J. Biol. Chem. 273, 16555–16560
23. Halio, S. B., Blumentals, I. I., Short, S. A., Merrill, B. M., and Kelly, R. M. (1996) J. Bacteriol. 178, 2605–2612
24. Du, X., Choi, I.-G., Kim, R., Wang, W., Janecak, J., Yokota, H., and Kim, S. H. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 14079–14084
25. Quigley, P. M., Korotkov, K., Baneyx, F., and Hol, W. G. J. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 3137–3142
26. Horvath, M. M., and Grishin, N. V. (2001) Proteins 42, 230–236
27. Hendrickson, W. A., Horton, J. R., and LeMaster, D. M. (1990) EMBO J. 9, 1665–1672
28. Otninowski, Z., and Minor, W. (1997) Methods Enzymol. 276, 307–326
29. Weeks, C. M., and Miller, R. (1999) J. Appl. Crystallogr. 32, 120–124
30. Terwilliger, T. C., and Berendzen, J. (1999) Acta Crystallogr. Sec. D 55, 849–861
31. Jones, T. A., Zou, J. Y., Cowan, S. W., and Kjeldgaard, M. (1991) Acta Crystallogr. Sec. A 47, 110–119
32. Jorg, G., Tao, X., Xu, Y., and Tong, L. (2001) Acta Crystallogr. Sec. D 57, 1127–1134
33. Brungart, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gies, P., Grosse-Kunstleve, R. W., Jiang, J.-S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonsen, T., and Warren, G. L. (1998) Acta Crystallogr. Sec. D 54, 905–921
34. Hendrickson, W. A. (1991) Science 254, 51–58
35. Rossmann, M. G. (1990) Acta Crystallogr. Sec. A 46, 73–82
36. Holm, L., and Sander, C. (1993) J. Mol. Biol. 233, 123–138
37. Bravo, J., Mate, M. J., Schneider, T., Switala, J., Wilson, K., Loewen, P. C., and Fita, I. (1999) Proteins 34, 155–166
38. Watt, W., Koeppler, K. A., Mildner, A. M., Heinrikson, R. L., Tomasselli, A. G., and Waterpaugh, K. D. (1999) Structure 7, 1135–1143
39. Carson, M. (1987) J. Mol. Graphics 5, 103–106
40. Nicholls, A., Sharp, K. A., and Honig, B. (1991) Proteins 11, 281–296
