Crystal Structures of Human DJ-1 and *Escherichia coli* Hsp31, Which Share an Evolutionarily Conserved Domain*

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Human DJ-1 and *Escherichia coli* Hsp31 belong to the ThiJ/PfpI family, whose members contain a conserved domain. DJ-1 is associated with autosomal recessive early onset parkinsonism and Hsp31 is a molecular chaperone. Structural comparisons between DJ-1, Hsp31, and an Archaea protease, a member of ThiJ/PfpI family, lead to the identification of the chaperone activity of DJ-1 and the proteolytic activity of Hsp31. Moreover, the comparisons provide insights into how the functional diversity is realized in proteins that share an evolutionarily conserved domain. On the basis of the chaperone activity the possible role of DJ-1 in the pathogenesis of Parkinson’s disease is discussed.

Completed or ongoing genome sequencing projects have added many members to protein families by finding out new proteins belonging to the families. ThiJ/PfpI family is such an expanding protein family whose members are evolutionarily distributed from Archaea to Eukarya. The family members share a domain (ThiJ domain) that is structurally related to type I glutamine amidotransferase domain (GAT1 domain) (1). GAT domains are found in a large group of biosynthetic enzymes that catalyze the transfer of water to free glutamine to release ammonia as a substrate for subsequent biosynthetic reactions at a contiguous synthase domain or subunit (2). Unlike the GAT domain, however, the biological role of ThiJ domain is unclear. PFAM (3) shows that ThiJ/PfpI family contains many hypothetical proteins. Even the family members with known functions have distinct activities. For example, the domain has activities such as regulation of RNA-protein interaction, phosphorylation of hydroxymethylpyrimidine, and proteolysis. Thus, deciphering the molecular role of the domain and the evolutionary relationship among the family members is a great challenge.

We have determined the crystal structures of *Escherichia coli* Hsp31 and human DJ-1, members of the ThiJ/PfpI family, as a start toward dissecting structural and functional relationships among the family members. Hsp31, the *yeito* gene product, is a molecular chaperone whose expression is induced by heat shock (4). Although the physiological role of Hsp31 has not been clearly established, the implication of Hsp31 in the protein quality control was discussed in the previous studies (5, 6). To control the protein quality, cells respond to a sudden increase of environmental temperature with the overexpression of heat shock proteins. During heat shock most of the heat shock proteins act either as molecular chaperones to assist unfolded proteins in folding or as proteases to degrade proteins that fail in refolding (7).

DJ-1, which is preferentially expressed in the testis and moderately in other tissues, was first identified as a novel candidate of the oncogene product that transformed mouse NIH3T3 cells with cooperation with activated ras (8). After the first identification, various physiological roles of DJ-1 were successively revealed. DJ-1 was characterized as a protein that regulates an RNA-protein interaction (9). DJ-1 is also related to sperm fertilization (10) and positively regulates the androgen receptor by impairing the binding of PIS (protein inhibitor of activated STAT xo) to the receptor (11). Remarkably, the association of DJ-1 with autosomal recessive early onset parkinsonism was recently demonstrated (12). In summary, DJ-1 has multiple functions whose correlation is presently obscure. Furthermore, it was reported that DJ-1 was expressed and that its pI was changed from 6.2 to 5.8 by treatment of cells with paraquat and endotoxin, which induce reactive oxygen species (13, 14), suggesting a function as an antioxidant protein. The transcription of YDR533C, a yeast DJ-1 homologue, is induced together with genes involved in the oxidative stress response (15). Here, we describe the crystal structures and biochemical data to provide an important framework for the elucidation of the molecular mechanisms of DJ-1 and Hsp31 functions.

EXPERIMENTAL PROCEDURES

Purification, Crystallization, and Structure Determination—Hsp31, C185A mutant of Hsp31 (referred to as C185A throughout the text), and SeHsp31 were purified as described (16). DJ-1 gene (encoding residues 1–189) was amplified by polymerase chain reaction from a human kidney cDNA library. The gene was inserted downstream of the T7 promoter on the expression plasmid pET-21a, and the plasmid was
introduced in E. coli strain B834 (DE3), a methionine auxotroph strain. Cells were grown to an A\textsubscript{600} of 0.6 in minimal media containing selenomethionine and 0.1 mg/ml ampicillin at 37 °C, and the expression of SeDJ-1 was induced by 0.5 mM isopropyl-\beta-D-thiogalactoside. After a 4-h induction at 37 °C, cells were harvested and resuspended in a 20 mM Tris buffer (pH 7.5) and disrupted by sonication. After centrifugation supernatants were loaded on Q-Sepharose Fast Flow column (Amersham Biosciences). The unbound fraction was consecutively loaded on SP-Sepharose Fast Flow column (Amersham Biosciences). The eluted fractions containing SeDJ-1 were collected and used for crystallization. Because SeDJ-1 was obtained in large quantity, we used SeDJ-1 for the studies presented here.

The previously reported orthorhombic crystals of Hsp31 (16) were not reproducible. Therefore, we found a new crystallization condition. Hsp31 was crystallized at 23 °C with mother liquids of 2 M ammonium sulfate and 0.1 M Tris-HCl (pH 8.5). Hexagonal crystals appeared within 1 week and belonged to space group P\textsuperscript{6}5\textsubscript{2}1\textsubscript{2}1. DJ-1 crystals (° monomer in asymmetric unit) were grown at 23 °C containing 1.4 M sodium citrate and 0.1 M HEPES (pH 7.5). For data collection crystals were frozen at 100 K using a cryostream cooler (Oxford Cryosystems, UK) after they were briefly immersed in a cryoprotectant solution containing 15% glycerol in the same mother liquor.

| Data sets, Space group | Hp31 | | | Dj-1 | P9\textsubscript{21} |
|---|---|---|---|---|---|
| Wavelength (Å) | 0.97924 | 0.9736 | 0.97156 | 1.2174 | 1.5418 |
| Resolution (Å) | 20-3 | 20-3 | 20-3 | 20-2.8 | 20-2.5 |
| Completeness (%)\textsuperscript{a} | 86.6 (89) | 86.1 (85) | 86.5 (89) | 99.1 (98.6) | 99.8 (99.1) |
| I/\sigma(I)\textsuperscript{b} | 11.6 (35.5) | 10.9 (32.4) | 10.5 (32.5) | 6.8 (16.7) | 6.1 (25) |
| Average I/σ (°) | 12.6 | 13.7 | 13.3 | 34.7 | 30 |
| Refinement statistics | | | | | |
| Resolution range (Å) | 2.8-2.5 | 2.8-2.5 | 2.8-2.5 | 2.8-2.5 | 2.8-2.5 |
| Number of reflections | 20-2.8 | 20-2.5 | 20-2.8 | 20-2.5 | 20-2.5 |
| Total number of atoms | 4,196 | 1,361 | 1,361 | 8,696 | 8,696 |
| Total water | 0 | 30 | 30 | 30 | 30 |
| Completeness of data (%) | 99.7 | 99.0 | 99.0 | 99.7 | 99.0 |
| R\textsuperscript{c} (R\textsuperscript{free}) (%) | 21.8 (27.7) | 22.2 (24.8) | 22.2 (24.8) | 22.2 (24.8) | 22.2 (24.8) |
| r.m.s. deviations\textsuperscript{d} | 0.007 | 0.01 | 0.01 | 1.422 | 1.41 |
| Angles (°) | | | | | |

\textsuperscript{a} The number in parentheses is for the outer shell.

\textsuperscript{b} R\textsubscript{sym} = \frac{\sum_{h,i} ||F_{h,i}|| - \sum_{h,i} ||F_{h,i}||}{\sum_{h,i} ||F_{h,i}||}, where \( I_{h,i} \) is the intensity of the i observations of symmetry related reflections of h.

\textsuperscript{c} R = \frac{\sum_{h,i} ||F_{h,i}|| - \sum_{h,i} ||F_{h,i}||}{\sum_{h,i} ||F_{h,i}||}, where \( F_{h,i} \) is the calculated structure factor from the atomic model and \( F_{h,i} \) is the calculated structure factor from the observed data.

\textsuperscript{d} Root mean square (r.m.s.) deviations in bond length and angles are the deviations from ideal values.

It was straightforward to solve the SeDJ-1 structure by using the phasing information derived from molecular replacement. SeDJ-1 has one more helix at the C terminus compared with PH1704. The electron density corresponding to the additional helix was apparent even in the first map, calculated by the molecular replacement solution. Thus the C-terminal helix was easily modeled. After completing side-chain replacement according to DJ-1 sequence, the model was subjected to a cycle of simulated annealing. The R-value dropped to 27.2%. At this stage, water molecules were added by using the X-solvate utility of QUANTA. The subsequent refinements and manual refitting of the model reduced R and R\textsuperscript{free} to 22.2 and 24.8%, respectively. The identity of the model stereochemistry was verified by PROCHECK (18). The Ramachandran plots indicate 94.2% (SeDJ-1) and 81.5% (Hsp31) of non-glycine residues are in the most favored regions, and all others except Cys-106 (SeDJ-1) and Cys-185 (Hsp31) are in the additionally allowed regions.

Prolate and Peptidase Assay—The degradation of BSA by DJ-1, Hsp31, and C185A were analyzed by SDS-PAGE. The assay buffer consists of 100 mM NaCl, 2 mM CaCl\textsubscript{2}, 10 mM dithiothreitol, and 20 mM Tris (pH 8.2). To change the pH of the reaction buffer we used 20 mM Tris (pH 7.5) and 20 mM Bicarbonate for pH 9.0. Peptidase activities were assayed by monitoring the production of AMC from the fluorogenic peptides Ala-AMC, Gly-AMC, Asp-AMC, Arg-AMC, Pro-AMC, Leu-AMC, Ser-AMC, Val-AMC, Tyr-AMC, Thr-AMC, Ile-AMC, Glu-AMC, Ala-Ala-Phe-AMC, and Ala-Leu-Lys-AMC (Sigma). 1 μg of Hsp31 was incubated for an hour with 100 μM substrate in 50 mM Tris (pH 7.5), 1 mM dithiothreitol, 5 mM Mg\textsubscript{2+}, and 1% Me\textsubscript{2}SO. AMC fluorescence (excitation at 380 nm, emission at 480 nm) was measured after the reaction was stopped by the addition of 200 μl of cold ethanol.

Chaperone Activity Assay—Chaperone activity was measured by monitoring the effect of the proteins on the thermal aggregation of CS (Sigma) and firefly luciferase (Roche Applied Science) at high temperatures. The mixture of CS and DJ-1 in the 40 mM HEPES (pH 7.5) buffer with 0.5 mM H\textsubscript{2}O\textsubscript{2} (or without H\textsubscript{2}O\textsubscript{2}) was preincubated for 3 min. For the luciferase assay firefly luciferase was diluted under excitation and 3 nm (emission), respectively. The molar ratio was
calculated on the basis of DJ-1 dimer, because DJ-1 contains one hydrophobic patch per dimer.

RESULTS AND DISCUSSION

Structures of DJ-1 and Hsp31—The tertiary structures of SeDJ-1\(^2\) and Hsp31\(^3\) adopt \(\alpha/\beta\) sandwich folds that are structurally aligned. Ribbon diagrams and C\(\alpha\) tracings of the structures are shown in Figs. 1 and 2. Hsp31 was previously reported as a dimer (5, 6), and DJ-1 was also found to be a dimer by size exclusion chromatography and dynamic light scattering (data not shown). Consistently, tight dimeric packing interactions were observed in the two protein crystals. In DJ-1 crystals, two DJ-1 monomers make extensive contacts, covering 35% of the molecular surface of each molecule. The dimeric interactions in DJ-1 are mainly made between helices (\(\alpha_1, \alpha_7, \) and \(\alpha_8\) from each monomer) and between strands (\(\beta_4\) from each monomer) (Fig. 1B). The interactions between \(\beta_4\)s from each monomer form an inter-molecular short sheet. A strong electron density was found in the dimerization site of Hsp31. Four histidine residues (His-86 on \(\alpha_2\) and His-123 on \(\alpha_3\) from each monomer) surround the strong electron density (Fig. 2, B and D). The fitting of a water molecule into the density remains a sparse \(F_o - F_e\) electron density, which clearly shows that the histidine residues coordinate a metal ion, not a water molecule. The metal is highly likely to be zinc because ICP-MASS reveals that metal binding in Hsp31. Furthermore, they failed to prove the proteolytic activity of Hsp31. 

Because one of the hallmarks of molecular chaperone function is the existence of hydrophobic patches on the surface. Chaperones interact with their nonnative substrate proteins via the surface hydrophobic stretches. In addition to the hydrophobicity, the hydrophobic patches interacting with unfolded proteins must have adequate geometric shape. The patches on the protruding regions are inadequate for the interactions with nonnative proteins. The hydrophobic patches in molecular chaperones are generally located in the grooves (21–23). The investigation of DJ-1 and Hsp31 structures reveals plausible hydrophobic patches that contain suitable chemical and geometric features for the interactions with nonnative proteins. The patch of DJ-1 is located in the shallow groove at the molecular interface (Fig. 1B), whereas Hsp31 contains a hydrophobic patch in each monomer (Fig. 2A). The hydrophobic patch of DJ-1 is formed mainly by two C-terminal helices (\(\alpha_7\) and \(\alpha_9\)) that tightly pack with each other (Fig. 1, B and D). A cluster of hydrophobic residues (Val-146, Phe-162, Leu-166, Ala-167, Ala-178, and Leu-187 from each monomer) is observed in the patch (Fig. 1B). In contrast to DJ-1, the N-terminal region (\(\beta_1, \beta_2, \) and \(\alpha_1\)) of Hsp31 is important in the formation of the hydrophobic patch (Fig. 2A). Two identical hydrophobic patches that consist of clusters of hydrophobic residues (Ala-14, Pro-21, Leu-26, Met-101, Tyr-106, Met-109, and Phe-120) are located diagonally on the surface of Hsp31 (Fig. 2C). The exposed hydrophobic residues on the hydrophobic patches in Hsp31 and DJ-1 are conserved or replaced by other hydrophobic amino acids compared with those in the homologues in other organisms (figure not shown). One exception is the A167K substitution in Drosophila DJ-1 homologue. It is noticeable that the long aliphatic chain of lysine is hydrophobic.

To confirm the function of the proposed hydrophobic patches, we designed a C-terminal deletion mutant of DJ-1 (A174–189, deletion of \(\alpha_8\)) and an N-terminal deletion mutant of Hsp31 (A1–33, deletion of \(\beta_1, \beta_2, \) and \(\alpha_1\)). Because the deleted terminal regions of both proteins are not engaged in the construction of the core structure of ThiJ domain (see “Peptidase Activity of Hsp31” below) (Figs. 2A and 4), the overall structures of the deletion mutants are expected to be conserved only with the disruption of the proposed hydrophobic patch. Consistently, circular dichroism revealed that the mutants are properly folded (data not shown).

The CS aggregation in the presence of the DJ-1 mutant increased about 25% compared with that in the presence of the same amount of wild type DJ-1 (figure not shown). The Hsp31 mutant was not able to facilitate the refolding of chemically denatured CS in contrast to wild type Hsp31 (figure not shown). These point to the importance of the terminal regions in the formation of the functional hydrophobic patches in the two proteins.

Peptidase Activity of Hsp31—Structural homologue search using the program DALI revealed that Pyrococcus horikoshii PH1704 and C-terminal ThiJ domain of E. coli PHII catalase (Cat_ThiJ) among ThiJ/PpI family members were structurally studied (24, 25). The structural comparison among four members (DJ-1, Hsp31, PH1704, and Cat_ThiJ) reveals an invariable core structure of ThiJ domain (Figs. 1A, 2A, and 4). The core structure consists of a central five-stranded parallel sheet and five helices packing onto either surface of the sheet (Figs. 1A, 2A, and 4). The core structure appears to tolerate large insertions since even the insertion of about 143 residues in Hsp31 does not disturb the core structure (Fig. 2A). As discussed later in this report, the tolerance to mutations is thought to be associated with the functional diversity of ThiJ domain.

Remarkably, a nucleophile elbow-like motif exists in the core (Fig. 4 and 5A). The nucleophile elbow is a distinctive strand-turn-helix motif that was first recognized in \(\alpha/\beta\) hydrolases. In an \(\alpha/\beta\) hydrolase, the nucleophile of a catalytic triad, either a

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\(^2\) The DJ-1 structures were reported when this manuscript was in the revision process (46–49). Although the structure was described exactly, neither insights nor data on the chaperone activity of DJ-1 were provided.

\(^3\) During the preparation of this manuscript the structure of Hsp31 was reported by another group (50). However, they did not detect the metal binding in Hsp31. Furthermore, they failed to prove the proteolytic activity of Hsp31.
Cysteine or a serine, takes a position in the turn of the nucleophile elbow. Sequence alignment in PFAM (3) shows that about 80% of the ThiJ/PfpI family members including DJ-1, Hsp31, and PH1704 have a conserved cysteine residue (Fig. 4). The structures of DJ-1, Hsp31, and PH1704 validate the location of the conserved cysteine (Cys-106 in DJ-1, Cys-185 in Hsp31, and Cys-100 in PH1704) in the turn of the nucleophile elbow (Fig. 5A). The structures also show that the $\psi/\phi$ angles for the potential nucleophile (the conserved cysteine) fall in an unfavorable region in the Ramachandran plot; this is the characteristic of the nucleophile in a nucleophile elbow.

Fig. 1. Structure of DJ-1. A, ribbon diagram of a DJ-1 monomer shown with secondary structures labeled. The core structure is represented by violet. Yellow indicates the insertion into the core structure. The boxed region indicates the dimerization site. The white arrow indicates the location of Leu-166. B, left, ribbon presentation of DJ-1 dimer. An ellipse indicates the location of the hydrophobic patch. Right, molecular surface representation showing the proposed hydrophobic patch. The molecular surface encompassing Val-146, Phe-162, Leu-166, Ala-167, Ala-178, and Leu-187 is colored yellow. The orientation is identical to that of left figure. C, stereo view of the superimposed Cα tracing of DJ-1 (yellow), Hsp31 (cyan), PH1704 (green), and Cat_ThiJ (pink). The orientation of the molecule is similar to A. The boxed region is the cap domain of Hsp31. A sphere indicates the location of the conserved cysteine residues. D, the $2F_o - F_c$ electron density maps contoured at 1$\sigma$ showing the region around Leu-166.

Fig. 2. Structure of Hsp31. A, ribbon diagram of a monomer shown with secondary structures labeled. The hydrophobic patch and the dimerization site are indicated by ellipses. The catalytic residues are drawn as green stick models. Green dots indicate hydrogen bonds between catalytic residues. The boxed region indicates the cap domain. The core structure of ThiJ domain is colored violet. The insertion into the core structure is represented by yellow. The orientation of the core is identical to Fig. 1A. B, ribbon presentation of Hsp31 dimer. Color scheme is identical to A. The catalytic residues and histidine residues involved in the metal binding are represented in balls-and-sticks. The box indicates the location of the metal binding site. The ellipses show the location of hydrophobic patches. C, surface representation of the Hsp31 dimer with the electrostatic potentials shown. Positive, hydrophobic, and negative electrostatic potentials are colored blue, white, and red, respectively. The orientation is identical to B. The ellipses indicate the location of hydrophobic patches. D, the $2F_o - F_c$ (black) and $F_o - F_c$ (red) electron density maps contoured at 1$\sigma$ and 3$\sigma$, respectively, showing the metal binding site.
The existence of nucleophile elbow containing a cysteine residue suggests an innate hydrolase activity of ThiJ domain. Consistently, PH1704 contains a proteolytic activity (25). However, no enzymatic activity of Cat_ThiJ has been reported where the conserved cysteine residue is substituted with a glycine. Intriguingly, in the superposed structures we observed that Hsp31 contains a Cys-185–His-186–Asp-214 triad that is structurally matched with the Cys-100–His-101–Glu-474 catalytic triad of PH1704 (Fig. 5B). Based on this observation we are convinced of the proteolytic activity of Hsp31, and as expected, Hsp31 that was previously reported to display no proteolytic activity (5, 6) degraded a folded protein substrate, BSA (Fig. 5C). In contrast, DJ-1 that has only His-126 at a distance of 3.7 Å from the conserved cysteine (Cys-106) without an acid residue (Fig. 5B) did not degrade BSA to the detectable level (Fig. 5C). The negligible proteolytic activity can be attributable to the incompletion of the catalytic triad in DJ-1. That is, the conserved cysteine residue in the nucleophile elbow does not ensure the hydrolase activity of ThiJ domain. For the hydrolase activity the full catalytic tools should be constructed. Thus, we cannot simply conclude that the ThiJ/PfpI family members with the conserved cysteine residue contain hydrolase activity.

The proteolytic activity of Hsp31 toward BSA is exceedingly weak, requiring 12 h at 37 °C to get approximately half of a 5-fold molar excess of BSA to show some level of degradation. The architecture of the putative active site in Hsp31 provides an explanation on the weak activity toward a protein substrate. The latent Cys-His-Asp triad of Hsp31 is accessible only through a small pocket with length of 14 Å (Fig. 6). The diameter of the pocket entrance is about 4 Å. The pocket size appears to confine the substrate spectrum of Hsp31 to peptides rather than protein substrates. Thus, we tested the peptidase activity of Hsp31 with synthetic fluorogenic substrates. As expected, Hsp31 showed peptidase activity displaying a specificity against two peptide substrates with small amino acids at N terminus (Ala-AMC and Gly-AMC) (Table II). Hsp31 was not able to cleave tripeptides like Ala-Ala-Phe-AMC, however, indicating that Hsp31 has a strong preference for very short peptides. Consequently, Hsp31 is highly likely to be a peptidase with short peptides as substrates, and thus, Hsp31 cannot efficiently degrade protein substrates.

The Possible Physiological Role of the Peptidase Activity of Hsp31—For the regulated protein breakdown, all organisms have developed machineries. In Thermoplasma acidophilum components of a complete proteolytic machinery have been identified; proteasome, tricorn protease, and three tricorn-interacting factors (F1, F2, and F3) (26). Proteasome (or HslUV in E. coli) produces peptides showing a broad size distribution with a peak length of 6–12 amino residues (27), sequentially tricorn turns over proteasomal products to peptides of 2–4 amino acid residues (28). Finally tricorn-interacting factors cleave any tricorn product to release single amino acids (29). Under heat shock, the proteolytic system actively works to degrade heat-damaged proteins that fail to refold by chaperones, and thus, short peptides that should be cleaved into...
single amino acids are produced in large quantity. In this situation, Hsp31 can play a role to cleave short peptides to produce single amino acids that are reusuable in the metabolic processes as tricorn-interacting factors do.

**Mutational Test of the Nucleophilic Activity of Cys-185 in Hsp31**—Based on the Cys-185–His-186-Asp-214 triad (Fig. 5B) and the proteolytic activity that is sensitive to the presence of reducing agent (Fig. 5C), Cys-185 can be assumed to be a nucleophile. To examine the nucleophilic activity of Cys-185, we designed a C185A mutant. The mutant did not cleave BSA as well as synthetic peptide substrates (Table II and Fig. 5C), suggesting that Cys-185 is the nucleophile of Hsp31.

**Crystal Structure of Oxidized DJ-1**—Because DJ-1 is converted to a variant with a more acidic pl in response to oxidative stimuli (13, 14), we determined the crystal structure of oxidized DJ-1 to reveal the cause of the variation. The structure of the oxidized DJ-1 is nearly identical to the non-oxidized structure except the modification of Cys-106. In the oxidized structure we observed additional electron density in the side-chain region of Cys-106 in the nucleophile elbow (Fig. 7). A sulfenic acid is well fitted into the additional electron density. A cysteine residue can be oxidized to sulfenic acid and further to sulfinic acid and cysteic acid under oxidizing environment (30). Because the pKa value of sulfenic acid and cysteic acid are both less than 2, the oxidation of the surface-exposed cysteine residue can lower the pl value of proteins (31). Thus, the reported lowering of DJ-1 pl in oxidizing conditions is apparently linked to the oxidation of Cys-106.

**DJ-1 and Parkinson’s Disease**—DJ-1 is the third protein to be identified as definitively involved in familial PD, the other two being α-synuclein and parkin. Therefore, the pathological role of DJ-1 in PD must be considered in conjunction with α-synuclein and parkin. α-Synuclein is a major component of Lewy body, which is a common pathological hallmark of PD (32). Parkin is an E2-dependent E3 protein-ubiquitin ligase that is implicated in the protein degradation pathway via proteasome system (33, 34). One common effect of the pathogenic mutations in α-synuclein and parkin is the accumulation of abnormal proteins. A shared property of α-synuclein mutants is that they form a protofibril, a kind of protein aggregation that can seed to form the amyloid fibrils present in Lewy body, more readily than wild type (32). Also, pathogenic mutations in parkin lead to proteasome dysfunction, resulting in the accumulation of proteins that are fated to be degraded (35). In neurodegenerative diseases including PD, the pathology and the eventual death of specific neuronal populations are closely correlated with the accumulation of abnormal proteins (36). It is now well established that the cellular protection mechanisms against the accumulation of abnormal proteins are deeply implicated in the progression of neurodegenerative diseases.

Because the chaperone machinery is the representative protection system against the accumulation of abnormal proteins in cells, the chaperone activity of DJ-1 suggests that DJ-1 plays a protective role in the pathogenesis of PD. Remarkably, the augmentation of a chaperone (Hsp70) activity in vivo suppressed α-synuclein neurotoxicity, whereas the interference of the chaperone function enhanced α-synuclein-induced neuronal loss in Drosophila model for PD (37). This implies that a

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**Table II**

| Substrates | Wild type | C185A |
|-----------|-----------|-------|
| Ala-AMC   | 20270.6 nmol/h/mg | 9.1 nmol/h/mg |
| Gly-AMC   | 7960.6 nmol/h/mg  | 0 nmol/h/mg   |

*The weak cleavage of Ala-AMC remained constant at various concentrations of C185A, indicating that the cleavage occurred not by C185A but by unknown processes. In the case of wild type, the cleavage of synthetic peptides increased according to the increment of Hsp31 concentration.*
Oxidized DJ-1

Fig. 7. Modification of a cysteine residue into a sulfenic acid under an oxidizing condition. The 2Fo – Fc electron density maps contoured at 1σ showing Cys-106 of DJ-1. The structure of oxidized DJ-1 was solved at 3.0 Å.

single chaperone can have serious effects on the progression of PD. Signs of oxidative stress were observed in post-mortem studies of PD brains (38, 39). DJ-1 is an oxidative stress-responsive protein (13, 14), and the structure and the chaperone activity of DJ-1 are maintained even in the presence of H2O2 (Figs. 3A and 7). Taken together, it is highly conceivable that DJ-1 plays a defensive role in the progression of PD. It is, thus, quite interesting to examine whether the overexpression of DJ-1 could suppress the toxic effects of mutations in α-synuclein or parkin in neuronal cells.

A point mutation in DJ-1 (L166P) was observed in PD patients (12). Leu-166 is located in α7, which is a major part of the hydrophobic patch (Fig. 1B). As Bonifati et al. (12) suggested, the mutation can destabilize the helix structure. The destabilization of α7 is then able to interfere with the tight packing between α7 and α8. Because α7 and α8 play critical roles in the patch construction, the breakage of the packing between the two helices is able to disrupt the hydrophobic patch (Fig. 1, B and D). Thus, in terms of the chaperone activity, L166P is likely to be a loss-of-function mutation.

Functional Diversity in Proteins Sharing ThiJ Domain—ThiJ/PfpI family is not a simple protease-chaperone family. ThiJ domain contains other biochemical activities besides the proteolytic activity and the chaperone activity. For example, some proteins with ThiJ domain are known to be involved in the thiamine biosynthesis (ThiJ; EC 2.7.1.49) and de novo synthesis of purine nucleotides (phosphoribosylformylglycinamidine synthase I (EC 2.7.1.49) from Sulfolobus solfataricus). Moreover, the identification of functions of hypothetical proteins that compose a large portion of ThiJ/PfpI family would reveal novel activities of ThiJ domain. An interesting question is, thus, what is the origin of the functional diversity of ThiJ domain spanning the whole kingdoms of life? The structural investigation of DJ-1, Hsp31, and PH1704 sheds light on the root of the diverse activities of ThiJ domain.

One of the structural features of the three proteins is the distinct quaternary structure despite the homologous tertiary structure. PH1704 forms a hexameric structure (25). The dimeric conformation of DJ-1 is also dissimilar to that of Hsp31 (Figs. 1B and 2B). The different surface properties induced by ad hoc mutations in ThiJ domain, which are easily appreciated by the disparity in the electrostatic and geometric surface features of ThiJ domains in the three proteins, are responsible for the generation of diverse oligomerization sites (Fig. 8). The biochemical activities of PH1704 and DJ-1 are closely correlated with their unique quaternary structures. An aspect of the catalytic triad in PH1704 is its formation based on the hexameric conformation; Cys-100 and His-101 form the triad with a glutamate residue (Glu-474) from an adjacent monomer at the molecular interface (25) (Fig. 5B). The dimer formation in DJ-1 creates a hydrophobic patch at the molecular interface instead of completing a catalytic triad (Fig. 1B), which defines DJ-1 as a chaperone.

In the case of Hsp31, the dimeric conformation seems to be independent of the construction of the functional sites. Instead, cap domain on top of ThiJ domain is dedicated to the formation of the functional sites. Cap domain completes the Cys-His-Asp triad by conferring Asp-214 on the triad, and the hydrophobic patch is also constructed on one side of the cap domain (Fig. 2A). The primary sequence comparison shows that Hsp31 contains ~110 residues more than DJ-1 and PH1704 (Fig. 4). Cap domain is composed of four strands (β1, β2, β4, and β5) and two helices (α1 and α8) that are contributed by the additional residues (Figs. 2A and 4). In summary, the functional sites of DJ-1, Hsp31, and PH1704 reside in the molecular interfaces or in the newly generated domain, not in the ThiJ domain itself. That is, the oligomerization and the generation of a new domain are strategies for producing the functional diversity of ThiJ domain.

Concluding Remarks—This report provides the first experimental demonstration of the chaperone activity of DJ-1 that has roles in various biological processes including the pathogenesis of PD. The protective roles of the chaperone machinery in the pathogenesis of neurodegenerative diseases like PD (37,
40–45) indicate that the ability of DJ-1 to suppress protein aggregations can be a defense tool in the pathogenesis of PD. We also describe the proteolytic activity of Hsp31 (a molecular chaperone) that was previously reported to contain no proteolytic activity. The Cys-185–His-186–Asp-214 triad and the effect of C185A mutation on the proteolytic activity indicate that Hsp31 is a cysteine protease. The disclosure of the proteolytic activity of Hsp31 gives a striking example of a stress-induced protein that is both a chaperone and a protease. Finally, the structures of DJ-1, Hsp31, and PH1704 reveal that the oligomerization or the generation of a new domain is employed to create the various biochemical activities of ThiJ/Ppi family members.

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