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Influence of Peptide Dipoles and Hydrogen Bonds on Reactive Cysteine pK_a Values in Fission Yeast DJ-1

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Abstract

Cysteine residues with depressed pK_a values are critical for the functions of many proteins. Several types of interactions can stabilize cysteine thiolate anions, including hydrogen bonds between thiol(ate)s and nearby residues as well as electrostatic interactions involving charged residues or dipoles. Dipolar stabilization of thiolates by peptide groups has been suggested to play a particularly important role near the N-termini of α -helices. Using a combination of X-ray crystallography, site-directed mutagenesis, and spectroscopic methods, we show that the reactive cysteine residue (Cys111) in *Schizosaccharomyces pombe* DJ-1 experiences a 0.6 unit depression of its thiol pK_a as a consequence of a hydrogen bond donated by a threonine sidechain (Thr114) to a nearby peptide carbonyl oxygen at the N-terminus of an α -helix. This extended hydrogen bonded interaction is consistent with a sum of dipoles model whereby the distal hydrogen bond polarizes and strengthens the direct hydrogen bond between the proximal amide hydrogen and the cysteine thiol(ate). Therefore, our results suggest that the local dipolar enhancement of hydrogen bonds can appreciably stabilize cysteine thiolate formation. However, the substitution of a valine residue with a proline at the i+3 position has only a minor effect (0.3 units) on the pK_a of Cys111. As proline has a reduced peptide dipole moment, this small effect suggests that a more extended helix macrodipolar effect does not play a major role in this system.

Keywords

cysteine pK_a; peptide dipole; DJ-1 superfamily; X-ray crystallography; redox biochemistry

Introduction

The ionization of cysteine to form a thiolate anion greatly increases the nucleophilicity of this residue and is critical for the functions of many proteins that contain cysteine in their active sites. The unperturbed solution pK_a value of the cysteine thiol is 8-9, which is higher than the pH of the cytosol. Consequently, proteins whose functions require cysteine thiolates must decrease the thiol pK_a value in order to render these cysteine residues reactive. The best established structural mechanism for depressing cysteine pK_a values is the donation of hydrogen bonds to the thiol(ate) [1]. Electrostatic stabilization of the thiolate using proximal cationic groups such as lysine, arginine, or protonated histidine sidechains can also contribute [2], although this is thought to be of secondary importance in most systems due to the flexibility of these sidechains [3, 4]. Despite well-understood general physical principles

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Structural models and diffraction data are available in the Protein Data Bank under the accession number(s) 4GDH (wild type SpDJ-1), 4GE0 (T114P SpDJ-1), and 4GE3 (T114V SpDJ-1).

of cysteine pK_a depression, the contribution of other protein structural features to cysteine ionization is more poorly understood.

Backbone peptide bonds could play an important role in cysteine pK_a depression, as these abundant groups have both a permanent dipole moment and can also donate a hydrogen bond to the thiolate. Therefore, correctly oriented peptide groups can lower the pK_a value of a cysteine, as has been observed in the thioredoxin family [5]. In the case of an α -helix, the vector sum of the partially aligned peptide dipoles along the helical axis has been proposed to result in a cumulative helical “macro-dipole” moment, with partial positive charge ($\sim +0.5e$) at the N-terminus and partial negative charge ($\sim -0.5e$) at the C-terminus [6, 7]. The α -helix macrodipole has been suggested as the explanation for pK_a perturbation of residues near helical termini [8], particularly the frequent occurrence of reactive cysteine residues near the N-termini of α -helices [9]. However, the magnitude and relevance of this effect have been called into question by computational work indicating that donation of hydrogen bonds by amide hydrogen atoms, rather than an electrostatic helix macrodipolar effect, is primarily responsible for the lowered pK_a values of residues at the N-termini of helices [4, 10]. Furthermore, solvent exposure of one or both ends of the helix can substantially reduce its effective macrodipole moment, although well-shielded helical termini can accumulate significant partial charges [11].

Experimentally addressing the influence of peptide groups on cysteine pK_a values in proteins has been hampered by the difficulty of creating mutations that alter peptide dipoles or protein backbone hydrogen bonds but do not perturb other aspects of the protein structure. Unlike simple amino acid substitutions that can be used to alter the charge or the hydrogen bonding potential of sidechains, no comparable experimental strategy exists for easily modifying peptide groups in proteins. Despite these difficulties, a detailed experimental study of the helical macrodipole effect in sperm whale myoglobin has been performed, suggesting that both peptide hydrogen bonding and the helical macrodipole effect contribute to lowering cysteine pK_a values [12]. Other experimental studies, however, have found little support for a contribution from the helical macrodipole, even when local interactions with peptides are thought to stabilize charged groups [13].

The DJ-1 superfamily is a functionally diverse collection of proteins containing a highly conserved cysteine residue that can function as a catalytic nucleophile [14-16] or a potential redox sensor [17] in various members. This cysteine residue is functionally critical in most characterized DJ-1 superfamily proteins, although rare exceptions that lack the cysteine exist [18]. Human DJ-1 is a disease-associated protein with multiple proposed functions in cytoprotection and mitochondrial function [19, 20]. The oxidation-sensitive cysteine (Cys106) in human DJ-1 has a depressed pK_a value of 5.4 due, in part, to an unusual hydrogen bond formed between the Cys106 thiolate and a protonated glutamic acid sidechain (Glu18) [21]. However, mutagenesis has shown that this hydrogen bond accounts for only ~ 1 unit of C106 pK_a depression, with the remaining ~ 2 units unexplained [21]. The reactive cysteine residue is located at the N-terminus of an α -helix, suggesting that hydrogen bonding or dipolar contributions from the helix may contribute to its low pK_a value. Problematically, an empirically-based computational method fails to accurately predict either the direction or magnitude of the reactive cysteine pK_a perturbation in DJ-1 [22], complicating the computational analysis of the structural determinants of cysteine reactivity in this superfamily. Because the DJ-1 superfamily is a varied group of proteins with a conserved reactive cysteine residue, it is an ideal system in which to investigate the significance of various structural contributions to cysteine pK_a modulation.

We have investigated the contribution of peptide groups to the lowering of cysteine pK_a values using a combination of structural and biochemical methods applied to a DJ-1

homologue from the fission yeast *Schizosaccharomyces pombe* (SPAC22E12.03c; SpDJ-1 hereafter). The pK_a value of the reactive cysteine (Cys111) in SpDJ-1 is ~1 unit lower than that of the highly homologous human protein due to subtle changes in the dipolar environment of the Cys111 thiol resulting from a rare Pro/Thr substitution near the N-terminal region of the α -helix that contains Cys111. Site-directed mutation of the Thr114 of SpDJ-1 indicates that hydrogen bond donation by the Thr sidechain to the proximal peptide depresses the pK_a of Cys111, suggesting that the interplay of hydrogen bonding and local dipolar effects can play a significant role in modulating the pK_a of reactive cysteine residues. In contrast, the helical macrodipole appears to exert little influence on the pK_a of Cys111.

Results

The oxidation-sensitive cysteine in SpDJ-1 has a lower thiol pK_a than in human DJ-1

The 1.05 Å resolution crystal structure of SpDJ-1 shows that the protein is highly similar to other DJ-1 homologues from human (1.2 Å monomer C α RMSD)[21] and *E. coli* (1.5 Å monomer C α RMSD) [23], as expected based on shared sequence identity [24]. Most of the structural differences between human and SpDJ-1 are located in solvent exposed parts of the protein, while the core backbone structures of these proteins are nearly identical (Fig. 1A). Like other DJ-1 proteins, SpDJ-1 contains a conserved cysteine residue (Cys111) that is easily oxidized to the cysteine-sulfinate (Cys-SO₂⁻) during crystallization (Fig 1B). Interestingly, we could only crystallize SpDJ-1 in its oxidized form, suggesting that formation of Cys111-SO₂⁻ facilitates crystallization by stabilizing the protein through the formation of hydrogen bonds with surrounding residues [25]. Because only the oxidized protein can be crystallized, analysis of the structural determinants of lowered cysteine pK_a values in SpDJ-1 is predicated on the assumption that there are no significant structural differences between the reduced and oxidized proteins. This assumption is supported by the nearly identical structures of oxidized and reduced human DJ-1 [20, 26], but it is an interpretative limitation that must be borne in mind throughout the structural analysis.

Previous studies of human DJ-1 have shown that the reactive cysteine residue has a low pK_a value of 5.4 [21], and we have observed that other DJ-1 homologues have similar cysteine pK_a values of ~5 (unpublished data). However, Cys111 in SpDJ-1 has a somewhat lower pK_a value of 4.60±0.04 as measured using UV spectrophotometry (Fig. 2). To determine the structural basis of this decreased pK_a value, we compared the environments of the reactive cysteine residues in these two proteins. In general, a very similar array of amino acids surrounds the reactive cysteine residues in human and SpDJ-1. The backbone amide hydrogen atom of Ala112 makes a putative hydrogen bond with the thiol(ate) sidechain of Cys111 with a N-S distance of 3.25 Å and a N-H-S angle of 127.8°. This hydrogen bond angle is not optimal, but it is within one standard deviation of the value of 143.5±24.6° calculated from experimentally determined structures containing backbone amide-thiol hydrogen bonds [27]. Therefore, the amide contributes a plausible hydrogen bond that may stabilize the ionization of Cys111, as noted in other proteins containing reactive cysteine residues at the N-termini of α -helices [1].

Residues in the first turn of the α -helix influence cysteine pK_a

Despite their similarities, a significant difference between human and SpDJ-1 is the substitution of a conserved proline in the human protein (P109 in human DJ-1 numbering) with a threonine in SpDJ-1 (T114 in SpDJ-1 numbering) in the first turn of the α -helix that contains Cys111 at its N-terminus. The sidechain hydroxyl group of T114 donates a hydrogen bond to the carbonyl oxygen of Cys111 in SpDJ-1, providing a direct structural coupling between these sites that could influence Cys111 (Fig. 3A). In addition, proline has

an intrinsically smaller peptide dipole moment due to the absence of an amide hydrogen atom, which diminishes the contribution of this residue to a postulated helical macrodipolar effect.

Site-directed mutagenesis was used to introduce a T114P mutation in SpDJ-1, which makes this protein more similar to other DJ-1 homologues (including human DJ-1) that contain a proline at this position. The 1.45 Å resolution crystal structure of T114P SpDJ-1 was determined in the same space group (P1) and lattice as the wild-type protein. The conservation of space group and lattice in crystals of wild-type and T114P SpDJ-1 minimizes any structural changes that might result from altered crystal packing contacts and thus facilitates the direct comparison of these structures. T114P is a structurally conservative mutation (overall Cα RMSD=0.15 Å with wild-type SpDJ-1) that causes small local structural changes (Fig. 3A), including an approximately 23° reorientation of the peptide N-H vector near Cys111 and the loss of the hydrogen bond donated by the sidechain of Thr114 (Fig 3B). The change in peptide orientation is due to the introduction of hydrogen atoms attached to the Cδ atom of Pro114, which would sterically clash with the backbone carbonyl oxygen of Cys111 in the wild-type protein and thus requires the slight displacement of this peptide (Fig 3A). The positions of the hydrogen atoms in all structures have been calculated using Molprobability [28], as hydrogen atoms could not be confidently located in the electron density maps for these structures.

The impact of the T114P mutation on the pK_a value of Cys111 was determined using UV spectroscopy to monitor thiolate formation (see Materials and Methods). The T114P substitution elevates the pK_a value of Cys111 from 4.60 to 5.54 ± 0.05 (Fig. 4), comparable to the pK_a of the corresponding cysteine in human DJ-1 (pK_a=5.4). This increase in Cys111 pK_a is larger than expected based on the modest structural changes resulting from the T114P mutation. The change in the orientation of the peptide group slightly increases the distance between the Cys111 Sγ atom and the amide hydrogen atom from 2.54 Å in the wild-type protein to 2.67 Å in T114P SpDJ-1. This 0.13 Å change in H-S distance would be expected to slightly weaken the hydrogen bond between the peptide and the thiol(ate), but not by enough to account for a nearly 1 unit increase in cysteine pK_a value.

An extended hydrogen bonding interaction with a proximal peptide group depresses cysteine pK_a

The T114P substitution results in three changes that could effect Cys111: (1) it removes a hydrogen bond between residue 114 and the peptide carbonyl oxygen of Cys111, (2) it results in the 23° reorientation of the peptide and a slight (0.13 Å) increase in H-S distance, and (3) it may reduce the helical macrodipole due to the smaller intrinsic peptide dipole moment of proline. In order to determine which of these candidates are major contributors, we made the T114V substitution in SpDJ-1. Because valine and threonine have sterically similar sidechains and the same peptide dipole moment magnitudes, only the hydrogen bond between residue 114 and Cys111 is expected to change as a result of the T114V substitution. The 1.5 Å resolution crystal structure of T114V SpDJ-1 in space group P1 shows that the valine substitution is highly structurally conservative (0.09 Å Cα RMSD with wild-type SpDJ-1) and results only in the loss of a hydrogen bond, with no change in peptide orientation compared to wild-type SpDJ-1 (Fig. 5A). Similar to T114P SpDJ-1, the T114V mutant has a Cys111 pK_a value of 5.23±0.08 (Fig. 4A), 0.6 units higher than wild-type SpDJ-1. Given the similar orientation of the Cys111-Ala112 peptide group in both wild-type and T114V SpDJ-1, our results indicate that the hydrogen bond between the sidechain of Thr114 and the peptide carbonyl oxygen atom proximal to Cys111 is a significant contributor to the depressed pK_a value of Cys111, even though this hydrogen bond is not made directly to the thiolate. However, the Cys111 pK_a value of the T114V mutant is still 0.3 units lower than that of T114P SpDJ-1, which places an upper limit on the possible

contributions of the reorientation of the peptide at Cys111 in T114P SpDJ-1 and of the peptide dipole moment of residue 114 to the pK_a value of Cys111.

Discussion

The contribution of peptide groups to protein electrostatics has been long appreciated, however their specific contribution to the pK_a values of ionizable groups is disputed [9, 10, 12, 29, 30]. Peptide groups can influence cysteine thiol pK_a values either by electrostatic interaction with the partial charges on peptide atoms or through hydrogen bonding interactions, which are restricted to shorter length scales (3-5 Å) and have partially covalent character. In the dipolar approximation, the peptide group's electrostatic field possesses a directional dependence that is described by its dipole moment, approximately 3.5 Debye. Consequently, electrostatic interactions between peptides and thiolates have favored geometries that are similar to those expected for hydrogen bonded interactions. We note that the dipolar approximation requires that the distance between the charges in the dipole be infinitesimally small compared to the distance between the dipole and other interacting groups, which is not strictly valid on hydrogen bond length scales. However, if the dipole approximation is applied to hydrogen bonds involving peptide groups, then dipole-charge interactions could contribute to the strength of hydrogen bonds between peptides and thiolates. Due to these considerations, formally separating dipolar and hydrogen bond contributions to thiolate stabilization on shorter length scales (3-5 Å) is not straightforward.

The critical distinction between dipolar and hydrogen bonded contributions to thiolate stabilization occurs on longer length scales ($d > 5$ Å), where hydrogen bonding is no longer possible but electrostatic interactions persist. The observation that reactive cysteine residues are frequently located near the N-termini of α -helices has led to the suggestion that a cumulative helical macrodipole [6, 7], comprising the vector sum of the individual peptide dipoles aligned along the helical axis, may electrostatically stabilize thiol ionization [9, 30]. However, some computational studies have suggested that only peptide dipoles in the first turn of the α -helix contribute significantly to pK_a depression [29], and that hydrogen bonds between the thiol(ate) and the peptide backbone may be a more significant contributor to the helical effect [1, 3, 10]. Other computational studies, however, find more evidence for a macrodipolar effect [31]. Performing experiments capable of directly testing these hypotheses has been stymied by the inherent problem of altering peptide moieties, which are fundamental to protein structure and thus cannot be changed using standard mutagenesis approaches.

In light of these limitations, SpDJ-1 provides an ideal system to study the impact of dipolar contributions to cysteine pK_a values, as it contains both a reactive cysteine residue (Cys111) at the N-terminus of an α -helix and a hydrogen bond between an amino acid sidechain (Thr114) and a backbone peptide group that is colinear with the helical axis. Therefore, we were able to alter this hydrogen bond by standard site-directed mutagenesis of Thr114 and then study the impact of this lost hydrogen bond-peptide interaction on the thiol pK_a value of Cys111. Our findings are broadly consistent with the proposal that only the first few amino acids of an α -helix contribute significantly to modulating cysteine pK_a values [29]. The helix containing Cys111 is significantly solvent exposed at both termini, suggesting that solvent reaction field screening likely minimizes any helical macrodipolar effect [11]. Furthermore, there was little difference between the Cys111 pK_a value for a proline or valine substitution at the i+3 position, despite the fact that these amino acids have different peptide dipole moments, indicating that the helix macrodipole is not a significant contributor to the low pK_a of Cys111.

Despite the absence of strong evidence for a helical macrodipolar effect, a surprising result of our study is that a hydrogen bond donated to a nearby peptide can have an appreciable effect on thiol ionization even though affected hydrogen bond is not made directly with the thiolate and does not alter the orientation of the peptide group. We found that sidechain hydrogen bonding between the hydroxyl group of Thr114 and the carbonyl oxygen of Cys111 depresses the pK_a of the thiol by ~ 0.6 units, which is a considerable decrease for an indirect interaction. For comparison, the established direct hydrogen bond between the protonated Glu18 sidechain and the thiol(ate) of Cys106 of human DJ-1 decreases the Cys106 pK_a value by 1.0 unit [21]. As there are no other observed structural changes that can explain the elevated Cys111 pK_a value in T114V SpDJ-1, we propose that this is a bone fide dipolar effect, supporting the view that the alignment of dipoles proximal to the thiol can significantly influence its ionization. In the case of SpDJ-1, we propose that the hydrogen bond between Thr114 and the Cys111-Ala112 peptide results in partial charge cancellation of the peptide carbonyl oxygen and the H γ atom of Thr114 (Fig. 5B). This partial charge cancellation effect at the peptide carbonyl oxygen atom may enhance the strength of the direct hydrogen bond between the amide hydrogen and the thiol(ate) (Fig. 5B), thereby resulting in a more substantial depression of Cys111 pK_a than expected for a purely electrostatic phenomenon involving partial charges.

Although our experiments are confined to a single example from the DJ-1 superfamily, there is strong evidence that dipolar enhancement of thiolate formation is a general phenomenon. For example, local peptide dipolar interactions have been proposed to contribute to the low catalytic cysteine pK_a values in protein tyrosine phosphatase 1B and the RNA triphosphatase domain of mRNA capping enzyme [32]. Interestingly, the same study [32] suggested that the threonine sidechain has an underappreciated role in modulating cysteine pK_a values in various systems, which is strongly supported by our results. Therefore, we propose that dipolar interactions, particularly when combined with hydrogen bonding, are a potentially common mechanism by which peptide groups can increase the reactivity of functionally important cysteine residues.

Materials and Methods

Protein expression, purification, and crystallization

The gene encoding *Schizosaccharomyces pombe* DJ-1 (SpDJ-1; SPAC22E12.03c) was synthesized by GeneArt with codon usage optimized for *E. coli* expression. The insert was cloned between the NdeI and XhoI restriction sites of the bacterial expression vector pET15b and transformed into *E. coli* strain BL21(DE3) (Novagen Merck KGaA, Darmstadt, Germany) for protein expression. The expressed protein bears a thrombin-cleavable N-terminal hexahistidine tag to facilitate purification by metal affinity chromatography. Cells growth, protein expression, and purification were performed as described previously [16]. The purified protein was concentrated to 21 mg/ml in storage buffer (25 mM HEPES pH=7.5, 100 mM KCl, 2 mM DTT) using stirred pressure cell and centrifugal concentrators with 10 kDa nominal molecular weight cutoffs. Protein concentration was determined by UV-visible spectrophotometry using a calculated extinction coefficient for SpDJ-1 at 280 nm (ϵ_{280}) of $24,535 \text{ M}^{-1} \text{ cm}^{-1}$ (Expasy). The purified protein ran as a single band on an overloaded SDS-PAGE gel stained with Biosafe Coomassie blue (Bio-Rad, Hercules, CA, USA). The concentrated protein was divided into 50-100 μl aliquots that were rapidly frozen in liquid nitrogen and stored at -80°C .

X-ray crystal structure determination

SpDJ-1 at 21 mg/ml in storage buffer was thawed on ice and centrifuged at $16,000\times g$ for five minutes to remove particulates. Initial crystallization conditions were determined using

sitting drop vapor equilibration with commercially available sparse matrix screens. Conditions delivering crystals were further optimized using hanging drop vapor equilibration. Rod-shaped crystals measuring $\sim 300 \mu\text{m} \times 100 \mu\text{m} \times 100 \mu\text{m}$ appeared after 2-3 days in drops containing 2 μL of protein and 2 μL of reservoir (26-32% PEG4000, 100 mM Tris-HCl pH=7.4-8.2, 200 mM MgCl_2). These crystals were cryoprotected by serial transfer into aliquots of the reservoir solution supplemented with ethylene glycol at concentrations that increased in 5% increments to a final concentration of 20% (v/v). The wild-type crystals were cryocooled in nylon loops by direct immersion into liquid nitrogen. Both T114P and T114V SpDJ-1 were crystallized in similar conditions, but crystal growth was nucleated by microseeding for T114P SpDJ-1 using an acetone-degreased cat whisker that was used to touch crystals of the wild-type protein and then transferred into equilibrated drops containing T114P SpDJ-1. Crystals of T114P and T114V SpDJ-1 were cryoprotected as described above for the wild-type protein, but were cryocooled by transfer to the nitrogen cryostream prior to data collection. Notably, only SpDJ-1 that had spontaneously oxidized at Cys111 could be crystallized. Repeated attempts to crystallize SpDJ-1 in more reducing conditions (where all solutions contained 10 mM DTT) produced only heavy protein precipitation both in sparse matrix screens and in the same conditions that delivered crystals of the oxidized protein. Oxidation of the reactive cysteine residue has been observed to thermally stabilize human and *Drosophila melanogaster* DJ-1 [25], and a similar stabilization of SpDJ-1 may account for the preferential crystallization of the oxidized protein.

X-ray diffraction data for wild-type SpDJ-1 were collected at the Advanced Photon Source (Argonne, IL), GM/CA-CAT beamline 23ID-D from a single crystal maintained at 110 K. The crystal was illuminated with incident X-rays of 16 KeV and data were recorded on a MARmosaic 300 CCD detector in high and low resolution passes. SpDJ-1 crystallizes in space group P1, requiring a reorientation of the crystal followed by a second pass of data collection in order to record high resolution data that would be lost to the blind region in this space group. Diffraction data for T114P and T114V SpDJ-1 were collected at 110 K using a Rigaku MicroMax-007 rotating copper anode X-ray generator operating at 40 kV, 20 mA and equipped with confocal Osmic Blue optics and a RaxisIV++ image plate detector. As T114P and T114L SpDJ-1 also crystallize in space group P1, diffraction data were collected from two crystals of each mutant protein and scaled together in order to obtain satisfactory completeness. All data were processed using HKL2000 [33] and final data statistics are reported in Table I.

Phases for SpDJ-1 were obtained by molecular replacement using human DJ-1 (PDB 2OR3; [21]) as a search model in PHENIX [34]. An unambiguous solution (final translation function Z-score=10.2) was obtained by searching for two dimers in the asymmetric unit, and the initial model was automatically built by PHENIX. The model was improved manually in Coot [35] and then refined against a maximum likelihood amplitude-based target function with geometric and B-factor restraints using Refmac5 [36] in the CCP4 suite [37]. A bulk solvent correction was used to allow inclusion of the low resolution data for all structures, and anisotropic atomic displacement parameters (ADPs) and riding hydrogen atoms were included in the structural model of the wild-type protein refined at 1.05 Å resolution. Wild-type SpDJ-1 was used as the initial rigid body refinement model for T114P and T114L SpDJ-1 in Refmac5. For these two lower resolution models, the translation-libration-screw (TLS) model for rigid body ADP refinement was used [38, 39]. In all refinements, 5% of the data were randomly chosen and sequestered for the calculation of R_{free} [40]. Final models were validated using Molprobity [28] and Coot [35] and their statistics are shown in Table I.

Spectrophotometric cysteine pK_a determination

The ionization of cysteine was monitored by measuring the absorption of the thiolate anion at 240 nm as a function of pH [41, 42]. The pH of a double buffer (10 mM sodium citrate, 10 mM sodium phosphate) containing protein at 13 μ M was adjusted by addition of 0.5–1.0 μ L aliquots of 0.5 N or 5.0 N NaOH. At each value, the absorbance at 240 and 280 nm was measured using a Cary50 spectrophotometer (Varian, Palo Alto, CA, USA), and the A₂₈₀ value was used to normalize the A₂₄₀ measurement to protein concentration in the cuvette. The pH of each sample was determined using an Orion micro pH electrode (Thermo Fisher, Waltham, MA USA) that had been calibrated at the beginning of the experiment. Measured absorbance values were converted to an extinction coefficient at 240 nm (ϵ_{240}) using a calculated ϵ_{280} value for SpDJ-1 of 24,535 M⁻¹ cm⁻¹ (Expasy). Experiments were performed in triplicate and the averaged data were fitted to a modified version of the Henderson-Hasselbalch equation in Prism (GraphPad Software). Reported errors are derived from the fit procedure.

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Abbreviations

ADP	anisotropic atomic displacement parameters
DTT	dithiothreitol
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
TLS	translation-libration-screw

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Figure 1.

Human and *S. pombe* DJ-1 are structurally similar and both contain a reactive cysteine residue. In panel A, the backbone traces of human (yellow) and SpDJ-1 (blue) are superimposed (Ca RMSD=1.7 Å for the dimers, 1.2 Å for monomers), illustrating the high degree of similarity. The dyad axis of the dimer is shown as a line with an ellipse at the end, and the N- and C-termini of one monomer are labeled. In panel B, the 1.05 Å resolution 2mFo-DFc electron density map is shown contoured at 1.0 σ (blue) and 4.7 σ (purple) in the environment of the conserved cysteine residue (Cys111). This cysteine is oxidized to a cysteine-sulfenic acid (Cys111-SO₂⁻) and the nearby glutamic acid (Glu16) has unequal distribution of electron density between the C δ and O ϵ 1/2 atoms at 4.7 σ , suggestive of sidechain protonation. Both phenomena are similar to observations in human DJ-1 [21].

Figure 2.

Cys111 in SpDJ-1 has a low pK_a value. The pK_a of Cys111 was determined using the increased absorbance of the thiolate anion at 240 nm. The difference extinction coefficient at 240 nm ($\Delta\epsilon_{240}$) is plotted on the ordinate, which is calculated by subtracting the lowest measured value of the extinction coefficient from all others. The data were measured in triplicate with mean values (filled circles) plotted and standard deviations indicated with error bars. The data were fitted using the Henderson-Hasselbalch equation in Prism (GraphPad Software). The $\Delta\epsilon_{240}$ for the C111S mutant (open circles) shows no transition, confirming that it is the ionization of Cys111 that is being monitored.

Figure 3.

Residue 114 interacts with the peptide of Cys111. In panel A, structures of wild-type SpDJ-1 (black) and the T114P mutant (blue) are superimposed, with the hydrogen bond between Thr114 and the peptide of Cys111 indicated as a dotted line. The hydrogen atoms on the C6 atom (shown in white) cause a steric conflict that results in displacement of the peptide. The rotation of the Cys111 peptide plane by 23° is shown in Panel B.

Figure 4.

Thr114 depresses the pK_a of Cys111. The pK_a of Cys111 was determined in the T114P (open squares) and T114V (filled squares) mutants by measuring the pH-dependent change in extinction coefficient at 240 nm. All measurements were made in triplicate with standard deviations shown using error bars. In both cases, loss of the hydrogen bond between residue 114 and the peptide carbonyl oxygen atom of Cys111 elevates the pK_a above the value of 4.6 for the wild-type protein (5.2 for T114V, 5.5 for T114P SpDJ-1).

Figure 5.

A threonine-peptide hydrogen bond stabilizes the Cys111 thiolate via dipolar interactions. In both panels, Cys111-SO₂⁻ is shown because the oxidized form of the protein is the only one that yielded crystals. In panel A, the structures of wild-type (black), T114P (blue), and T114V (orange) are superimposed, showing that the T114V mutant only eliminates the hydrogen bond, with no other structural changes resulting from this mutation. In panel B, the partial charges and approximate peptide dipole moment in the region of Cys111 are shown. The hydrogen bond donated by Thr114 to the peptide of Cys111 is proposed to play an important role in enhancing the pK_a depression of the thiolate by altering the effective dipole moment of the peptide group through partial charge cancellation at the carboxyl oxygen atom.

Table 1
Data collection and refinement statistics

	Wild-type	T114P	T114V
Data collection			
X-ray source	APS 23ID-D	Rotating anode	Rotating anode
X-ray wavelength (Å)	0.78	1.54	1.54
Space group	P1	P1	P1
Cell dimensions			
<i>a</i> , <i>b</i> , <i>c</i> (Å)	44.51, 51.91, 82.71	44.49, 51.74, 82.75	44.58, 52.08, 82.91
α , β , γ (deg.)	89.07, 88.98, 66.84	89.10, 89.08, 66.86	89.01, 88.98, 66.55
Resolution (Å)	82.7-1.05	36.5-1.45	82.9-1.50
$R_{\text{merge}}^{a,b}$	0.075 (0.565)	0.066 (0.206)	0.072 (0.253)
$\langle I \rangle / \langle \sigma(I) \rangle^a$	21.6 (2.1)	19.9 (5.8)	16.6 (5.9)
Completeness ^a (%)	97.3 (90.2)	94.5 (84.9)	93.4 (87.6)
Multiplicity ^a	5.8 (3.9)	5.1 (3.6)	5.4 (4.4)
Refinement			
PDB code	4GDH	4GE0	4GE3
Program	Refmac5	Refmac5	Refmac5
Resolution (Å)	82-1.05	36-1.45	83-1.50
No. reflections	308911	113457	102117
R_{work} (%) ^c	11.9	17.9	15.8
R_{free} (%) ^d	14.3	20.8	18.9
R_{all} (%) ^e	12.0	18.0	16.0
No. Protein residues	763	760	764
No. Water atoms	898	928	875
B_{eq} factors (Å ²)			
Protein	13.9	18.1	17.7
Water	33.0	30.0	29.3
R.m.s. deviations			
Bond lengths (Å)	0.012	0.009	0.014
Bond angle (deg.)	1.55	1.24	1.50

^aValues in parentheses are for highest-resolution shell.

$R_{\text{merge}} = \frac{\sum_{hkl} \sum_i |I_{hkl}^i - \langle I_{hkl} \rangle|}{\sum_{hkl} \sum_i I_{hkl}^i}$, where *i* is the *i*th observation of a reflection with indices *h,k,l* and angle brackets indicate the average over all *i* observations.

$R_{work} = \sum_{hkl} |F_{hkl}^o - F_{hkl}^c| / \sum_{hkl} F_{hkl}^o$, where F_{hkl}^c is the calculated structure factor amplitude with index h,k,l and F_{hkl}^o is the observed structure factor amplitude with index h,k,l.

R_{free}^d is calculated as R_{work} , where the F_{hkl}^o are taken from a test set comprising 5% of the data that were excluded from the refinement.

R_{all}^e is calculated as R_{work} , where the F_{hkl}^o include all measured data (including the R_{free} test set).