For the article “Gene evolution at the ends of wheat chromosomes,” by Deven R. See, Steven Brooks, James C. Nelson, Gina Brown-Guedira, Bernd Friebe, and Bikram S. Gill, which appeared in issue 11, March 14, 2006, of Proc. Natl. Acad. Sci. USA (103, 4162–4167; first published March 6, 2006; 10.1073/pnas.0508942102), the authors note that in Fig. 4, the genome designations for the chromosomes incorrectly appeared as A, B, and C, due to a printer’s error. The corrected figure and its legend appear below.

**Fig. 4.** Distribution trend in NSH wESTs in deletion bins along chromosome arms. Bars represent negative log$_{10}$ of $P$ values resulting from a $\chi^2$ test for each bin that tested the null hypothesis that the proportion, with respect to that chromosome arm, of NSHs mapping to that bin is equal to that of all ESTs mapping to that bin. Bars extend to right for bins in which NSH frequency exceeds expectation and to left for those in which it is lower than expectation (asterisks indicate that the bin contained no observed NSHs).

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For the article “Structural basis for conformational plasticity of the Parkinson’s disease-associated ubiquitin hydrolase UCH-L1,” by Chittaranjan Das, Quyen Q. Hoang, Cheryl A. Kreinbring, Sarah J. Luchansky, Robin K. Meray, Soumya S. Ray, Peter T. Lansbury, Dagmar Ringe, and Gregory A. Petsko, which appeared in issue 12, March 21, 2006, of Proc. Natl. Acad. Sci. USA (103, 4675–4680; first published March 13, 2006; 10.1073/pnas.0510403103), the authors note that Fig. 1 appeared incorrectly. The corrected figure and its legend appear below. This error does not affect the conclusions of the article.

Fig. 1. Sequence alignment of UCH-L1 enzymes. Structure-based sequence alignment of UCH-L1 from different species is shown: HOM, Homo sapiens; MAC, Macaca fascicularis; SUS, Sus scrofa; EQU, Equus caballus; CAN, Canis familiaris; RAT, Rattus norvegicus; MUS, Mus musculus; TAE, Taeniopygia guttata; BUF, Bufo gargarizans; ACA, Acanthogobius flavimanus; ORE, Oreochromis niloticus; DAN, Danio rerio. The secondary structure elements of human UCH-L1 are indicated above the primary sequences, and conserved residues are highlighted (green, red, yellow, orange, and gray indicate conserved, hydrophobic, acidic, cyteine, polar, and glycine residues, respectively). Positions are identified as conserved if >80% of the residues are identical, or similar if hydrophobic in nature. Δ, catalytic triad; ε, oxyanion hole; ψ, H bonding with catalytic H161; γ, ubiquitin binding surface; η, P′ cleft; #, mutation site that reduced susceptibility to PD; *, mutation site that has been reported to cause familial PD; ~, the loop spanning the active site. Mammalian sequences are boxed.

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Structural basis for conformational plasticity of the Parkinson’s disease-associated ubiquitin hydrolase UCH-L1

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The ubiquitin C-terminal hydrolase UCH-L1 (PGP9.5) comprises >1% of total brain protein but is almost absent from other tissues [Wilkinson, K. D., et al. (1989) Science 246, 670–673]. Mutations in the UCH-L1 gene have been reported to be linked to susceptibility to and protection from Parkinson’s disease [Leroy, E., et al. (1998) Nature 395, 451–452; Maraganore, D. M., et al. (1999) Neurology 53, 1858–1860]. Abnormal overexpression of UCH-L1 has been shown to correlate with several forms of cancer [Hibi, K., et al. (1998) Cancer Res. 58, 5690–5694]. Because the amino acid sequence of UCH-L1 is similar to that of other ubiquitin C-terminal hydrolases, including the ubiquitously expressed UCH-L3, which appear to be unconnected to neurodegenerative disease, the structure of UCH-L1 and the effects of disease associated mutations on the structure and function are of considerable importance. We have determined the three-dimensional structure of human UCH-L1 at 2.4-Å resolution by x-ray crystallography. The overall fold resembles that of other ubiquitin hydrolases, including UCH-L3, but there are a number of significant differences. In particular, the geometry of the catalytic residues in the active site of UCH-L1 is distorted in such a way that the hydrolytic activity would appear to be impossible without substrate induced conformational rearrangements.

neurodegeneration | ubiquitination | ubiquitin ligase | x-ray crystallography

Cova lent conjugation of ubiquitin to proteins plays a crucial role in a wide variety of biological processes (1). Ubiquitin is a 76-aa protein that, as its name implies, is ubiquitously distributed and highly conserved throughout eukaryotic organisms. Ubiquitin is covalently linked to proteins via formation of an amide bond between the C-terminal carboxyl group of ubiquitin and the ε-amino group of a lysine residue on the acceptor protein.

Ubiquitination is reversible: ubiquitin is recycled by proteolytic removal from its conjugating protein by deubiquitinating enzymes (DUBs), a family of proteases with exquisite specificity for ubiquitinated substrates (2). These enzymes catalyze the hydrolysis of the amide bond C-terminal to the ubiquitin moiety, resulting in its release from protein adducts. Deubiquitination is widely recognized as an important component of regulatory mechanisms in all ubiquitin-dependent pathways. DUBs have been implicated in fundamentally important biological processes, including cell growth, differentiation, oncogenesis, development, and the regulation of chromosome structure (3). The DUB family consists of at least four distinct subfamilies: the ubiquitin C-terminal hydrolases (UCHs), ubiquitin-specific processing proteases, the OTU domain-containing enzymes, and the Jab/Csn5 and MPN domain-containing proteases. Members of the first three classes are cysteine proteases, whereas the fourth class is a group of metalloenzymes (4). Although a large number of mammalian sequences have been identified that could potentially code for a DUB, suggesting a wide substrate repertoire, very few of them have been assigned specific functions.

UCH-L1 is a small (molecular mass 24.8 kDa) acidic (pI 5.3) protein of unknown function that has been implicated both in Parkinson’s disease (PD) and in lung cancer. UCH-L1 is highly abundant in brain, constituting up to 2% of total protein (5). It is normally expressed exclusively in neurons and testis; however, abnormal expression of UCH-L1 is found in many primary lung tumors, lung tumor cell lines, and colorectal cancer (6–8).

UCH-L1 is similar to UCH-L3, the only structurally characterized mammalian DUB belonging to the UCH family, sharing 51% sequence identity and the conserved catalytic residues (9, 10) (Fig. 1). Biochemical studies have shown that both of these enzymes are catalytically active toward small ester- and amide-conjugates of ubiquitin (11). However, the tissue-specific expression pattern of these enzymes (unlike UCH-L1, UCH-L3 is uniformly expressed in all tissues, including brain) may suggest distinct sets of in vivo substrates. Moreover, the in vitro hydrolytic activities of the two enzymes are very different; UCH-L3 is >700-fold more active toward a model ubiquitin substrate (12). UCH-L1 has also been shown to have in vivo ligase activity, and this ligase activity is correlated with dimerization of the enzyme (12).

We are interested in the role of UCH-L1 in neurodegeneration in general and PD in particular. Shortly after the discovery of a link between a mutation in the α-synuclein gene with the PARK1 form of familial PD (13), the first association of genetics with PD, a substitution mutation (I93M) in the UCH-L1 gene was reported to be associated with an autosomal-dominant form of PD (14). Subsequently, another mutation (S18Y) was reported to be protective against PD (15). The magnitude of contribution made by these mutations to the etiology of PD is yet to be determined. However, it seems clear that UCH-L1 is important for the normal functioning of neurons. The loss of function of UCH-L1 in mice by in-frame deletion of exons 7 and 8 confers the gracile axonal dystrophy (gad) phenotype, which is characterized by accumulation of amyloid β-protein and ubiquitin-positive deposits along the neurons in sensory and motor nervous systems in the animal (16). Together with the fact that UCH-L1 is specifically expressed in neurons in large amounts, this observation suggests that UCH-L1 has an important functional role in the biology of the mammalian central nervous system (CNS).

Association of UCH-L1 with human diseases has sparked great interest in the enzyme, yet many of its important properties have not been determined. Its biological function and specific roles in human neurodegeneration | ubiquitination | ubiquitin ligase | x-ray crystallography

Conflict of interest statement: No conflicts declared.

Abbreviations: DUB, deubiquitinating enzyme; PD, Parkinson’s disease; PDB, Protein Data Bank; UCH, ubiquitin C-terminal hydrolase.

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disease are unknown, as are its substrates. Also unknown is whether it is a true enzyme, because all of the activities detected in vitro are significantly lower than that of any other known ubiquitin hydrolase or ligase (12). Here, we report the crystal structure of UCH-L1. The structure supports the idea that UCH-L1 is a tightly regulated enzyme and suggests an enzymatic activation mediated by substrate binding.

Results and Discussion

Overall Architecture of UCH-L1. The structure of UCH-L1 was determined by the molecular replacement technique (17) by using a homology model of UCH-L1 created from the atomic coordinates of its distant relative UCH-L3 (9) as a search model. The electron density map generated by using the molecular replacement model after rigid body refinement was of such quality that 70% of the protein structure could be readily fitted. The remainder of the structure was built during the course of refinement. The structure was built during the course of refinement. The structure currently has been refined to a crystallographic R factor of 22.2% and an Rpfree of 27.5% by using all WT data in the resolution shell 41.9–2.4 Å (see Table 1, which is published as supporting information on the PNAS web site). The model contains the complete 223-residue protein chain for both monomers (the 5 N-terminal residual residues that were carried over from the GST-tagged cloning vector were disordered and, therefore, not modeled). UCH-L1 with this N-terminal extension has the same level of in vitro activity against Ub-AMC as UCH-L1 without the extension (see Table 2, which is published as supporting information on the PNAS web site), 4 chloride ions, and 107 ordered solvent molecules. More than 98% of the nonglycine residues are placed within the most favorable and additionally allowed regions of the Ramachandran plot, and 1% are located in the disallowed areas, as defined within the program PROCHECK (18).

Although UCH-L1 is normally considered to be monomeric, there is evidence that it can dimerize in vitro and, that, when it does, it demonstrates a ubiquitin ligase activity in addition to its hydrolase activity (12). No other protein in the UCH family has been reported to dimerize. Therefore, the fact that the asymmetric unit contained two copies of the protein was of considerable interest. The noncrystallographic dimer is not a simple symmetrical arrangement of protomers related by a 2-fold rotation axis (180°), as is commonly found for homodimeric proteins. The two UCH-L1 monomers are related by a rotation of 161°. This unusual symmetry begs the question of whether this dimer could exist in solution. In the asymmetric unit, the dimer has a contact patch of 974 Å2, representing 9% of the total surface area of each monomer. This figure is within the range of that typically observed for stable oligomeric
contacts (see below) (19). We did not find any more extensive contacts with other molecules in the unit cell.

The UCH-L1 monomer is composed of two lobes, one consisting of five α-helices (α1, α3, α4, α5, and α6), hereafter referred to as the right lobe, and the other consisting of two helices (α2 and α7) and six β-strands (β1–β6), hereafter referred to as the left lobe. These secondary structures together form a helix–β-helix sandwich fold. Between the two lobes is a cleft in which the catalytic cysteine C90 (as determined by sequence similarity with other known UCH proteins) is located (Figs. 2B and 3B). The overall architecture of UCH-L1 closely resembles that of UCH-L3 and the yeast ubiquitin C-terminal hydrolase, Yuh1 (9, 20). The active-site cleft is composed of three secondary structure elements: a helix (α3), a strand (β3), and a loop (L9) on which the members of the putative catalytic triad C90, H161, and D176 reside, respectively. These three elements form a triangular arrangement and make extensive hydrophobic contacts within their respective lobes; however, there are only modest contacts between them. In the center of this triangle resides a column of four tightly bound water molecules, W1, W2, W3, and W4, forming H-bond bridges between α3 and L9, α3 and β3, β3 and L9, and between all three structures, respectively.

The column of four water molecules forms a wedge that penetrates 8.9 Å into the core of the protein and splits H161 8.2 Å apart from C90, disrupting the classical catalytic His-Cys diad (Fig. 4 A and C). H161 is held rigidly into this extruded position by an H-bonding network consisting of E60, N159, H161, D176, and R178 (Fig. 4B). The putative oxyanion hole would reside between C90, N88, and Q84 and closely resembles the oxyanion hole of papains (21) and UCH-L3 (9). We unexpectedly found a chloride ion occupying the oxyanion hole. This ion makes extensive electrostatic interactions with C90, N88, Q84, and with two water molecules (Fig. 4D). Activity assay of UCH-L1 withUb-AMC model substrate showed that chloride has no significant effect on the activity of UCH-L1 (R. Stein, personal communication). The water molecules W3 and W4 and the chloride ion are not found in the structures of UCH-L1 homologues UCH-L3 and Yuh1. The H-bonding network involving E60, N159, H161, D176, and R178 is also absent in the structures of these homologues, which have their active sites as blueprints of a classical catalytic triad. These differences may explain the observed low in vitro activity of UCH-L1 in comparison to its homologues. The biological relevance of these described differences between UCH-L1 and its homologues is unclear, but the structural differences might translate into differences in function or regulation. Interestingly, it has been shown that UCH-L1 and UCH-L3 function as reciprocal modulators of germ cell apoptosis in testis of mice (22). Furthermore, the difference in phenotype between the gracile axonal dystrophy (gad) mouse (UCH-L1 knockout equivalent) and the UCH-L3 knockout suggests that these proteins may have different functions (23).

The active-site cleft is covered by a loop, L8, spanning the cleft and positioned directly over C90. This loop forms a tunnel with the largest diameter of ~10 Å. Immediately to either side of the active site cleft, and along the interface of the two lobes, are the probable locations of the ubiquitin binding site (P site) and its unknown protein conjugate binding site (P’ site). The surface of the P site is acidic and consists of 7 conserved solvent-exposed acidic (E7, E11, D30, E35, and E37) and polar residues (N9 and S215). The P’ site resembles a v-shaped trough with loop L5 of the right lobe lining the right side and helix α2 of the left lobe lining the left side of the trough. In the middle of the trough resides a pit (P’ pit), which is surrounded by the conserved residues K83, T85, K131, and E134 (Fig. 3B).

Another noteworthy feature is the relatively flat surface on the outer face of the left lobe that consists of a cluster of conserved positively charged residues (H59, R63, H185, K199, R202, and R207). In this surface resides an extensive cavity (Fig. 3 C and D). These surface features are characteristic of a protein–protein interacting site, but whether this region has any significance to the biological function of UCH-L1 is not known. The equivalent region

Fig. 3. Molecular surface of UCH-L1. Conserved acidic side chains are colored red, basic side chains are colored blue, polar side chains are colored orange, and all nonconserved residues according to Fig. 1 are colored gray. B is related to A by a rotation of 50° about the y axis. D is related to C by a rotation of 90° about the x axis.
of UCH-L3 also presents an extensive surface, although not as flat, and lacks the cluster of conserved positively charged residues.

**Dimeric and Monomeric States.** To investigate whether the dimer complex in our crystal structure might exist in solution, we performed sedimentation equilibrium (SE) experiments under the same conditions in which we prepared the protein for crystallization. The results are consistent with UCH-L1 existing as monomer in solution under the conditions of our experiment. The SE data for the S18Y mutant are also consistent with a monomeric state (Fig. 6a and b, which is published as supporting information on the PNAS web site). Thus, at present, there is no indication that the dimer observed in this crystal structure exists in solution. The possibility of the N-terminal extension carried over from the cloning vector interfering with dimerization of UCH-L1 as observed by Liu et al. (12) is under investigation.

**P and P' Sites of UCH-L1.** Superposition of UCH-L1 with structures of its distant relative UCH-L3 (9) showed that the P site of UCH-L1 closely resembles that of UCH-L3 (Fig. 5b). Therefore, we expect that ubiquitin binds to UCH-L1 in the same configuration as it does to UCH-L3. Based on this expectation, we docked a model of ubiquitin to our structure of UCH-L1 based on the recently reported structure of UCH-L3 covalently bound to the ubiquitin analog UbVMe (24) (Fig. 7, which is published as supporting information on the PNAS web site). This docked model predicts that ubiquitin makes modest contact with UCH-L1 and that the interaction is mainly electrostatic in nature, contributed by conserved acidic residues (E7, E11, D30, E35, and E37) (Fig. 1).

Viewing down the active site of a surface rendering, UCH-L1 resembles a pie with a triangular slice cut out of it (Fig. 3a). This triangular void is the P' site. It is convenient to speculate that whatever binds there would resemble the missing piece of this pie.

Furthermore, within this triangular void resides the P' pit that is surrounded by four conserved residues, as mentioned above. It is easy to imagine that this pit can be used for selectivity and orientation of the protein substrate. Overall, the P' site resembles a female Lego block where the missing piece of the pie is the complementary male block. The P' site of UCH-L1 is more narrow than that of UCH-L3, because of helix α2 on the left bank of the trough being ~3 Å closer to the center than in UCH-L3. Also in contrast to UCH-L3, the shift of helix α2 has brought the carboxyl oxygen on the side chain of E60 within 2.5 Å of the imidazolium nitrogen of H161 (Figs. 4b and 5a and c). This interaction stabilizes the observed nonproductive structure of the catalytic triad, in which H161 is 8.7 Å apart from C90 as mentioned above. Because helix α2 is on the surface of the P' site, binding of anything sizable into this trough would potentially push it outward, breaking the H bonding between E60 and H161 and freeing H161 to come closer to C90 to complete the formation of an active catalytic triad.

**Active Site and Loop L8 of UCH-L1.** As presented in Fig. 4c, UCH-L1 cannot be a productive enzyme, at least not in the sense of traditional cysteine proteases where the minimum requirement for activity is a His-Cys diad. In the structure of UCH-L1, the distance of 8.2 Å between H161 and C90 is too long for any interactions that would produce a productive diad. In between these two residues resides a tightly bound water molecule, W4, which is 4.9 Å from H161 and 3.9 Å from C90. W4 forms stable H bonding with the backbone oxygen of F160 and a side-chain nitrogen of R178, with distances of 2.7 Å and 2.5 Å, respectively, and an angle of 93° (Fig. 4a). Because water W4 is close to C90, there is a possibility that this water may serve as a general base to abstract the proton from C90, thereby activating the enzyme in the absence of any contribution from histidine. However, water is a very weak base (pKₐ = 15.7), and there is nothing surrounding this water that could make it a significantly better base; therefore, we believe that H161 needs to undergo a conformational change that would bring it in close proximity to C90 for the enzyme to be active.
In order for H161 to move to the position required to form a productive catalytic triad, strand β3 and the side chain of H161 must move 1.7 Å and 3.6 Å, respectively, closer to helix α3 without changing the position of the immediate structures of helix α3 and loop L9. This unusual movement requires a large degree of plasticity in the noncovalent bonds that hold these three structures together. This plasticity could be provided, at least in part, by the water-bridge H bonds between these three structural segments (Fig. 4a). The innermost water molecule W1 bridges helix α3 to loop L9 with distances of 2.8 Å and 2.9 Å, respectively, with an angle of 113°. Water molecule W2 bridges helix α3 to strand β3 with distances of 3.0 Å and 2.7 Å, respectively, with an angle of 116°. These two water molecules are buried in the core of the protein and equivalent water molecules also exist in the structures of UCH-L3 (9), UCH-L3–UbVMe complex (24), and in Yuh1-Ubal (20). Therefore, they are likely to be structural water molecules that reside permanently in their positions.

The other two water molecules in the middle of UCH-L1 have no counterparts in UCH-L3 or any other ubiquitin hydrolase. Water W3 bridges loop L9 to strand β3 with distances of 2.8 Å and 2.9 Å, respectively, with an angle of 113°. This water molecule is also buried; however, it is H-bonded to the side chain of D176 (the equivalent residue in UCH-L3 forms part of the catalytic triad; ref. 9). Water W4 is within H-bonding distances of all three active-site structural elements, helix α3, strand β-3, and loop L9, with distances of 3.9 Å, 2.7 Å, and 2.5 Å, and angles of 86° and 93°, respectively. This water molecule is exposed to the surface and is positioned between H161 and C90, in exactly the place where the imidazolium nitrogen of H161 needs to be to form a productive catalytic triad. It appears that waters W3 and W4 must be displaced to generate an active enzyme. We speculate that the function of these waters is to hold the active site loosely together, keeping the enzyme in an inactivated state. The known flexibility of water-mediated H bonds would then provide the plasticity for the structure to collapse into a productive catalytic triad upon activation, whatever the trigger may be.

Except for the mispositioning of the catalytic histididine, the active site cleft of UCH-L1 generally resembles that of typical cysteine proteases such as those of papains (25). An added complexity in the active site of UCH-L1 is the loop L8 that covers the active site cleft (Fig. 2a). Loop L8 is an extended strand and strings across the active site. In this enclosed configuration, the only route of entry into the active site would be for the ubiquitinated substrate to tunnel in through the small aperture (≈10 Å) between the cleft and the loop. This configuration would restrict the size of the moieties attached on the C terminus of ubiquitin (P’ ligand) to <10 Å in diameter and would completely exclude any folded protein as UCH-L1’s P’ ligand.

Alternatively, on substrate binding, loop L8 may be displaced away from the active site, rendering it a typical accessible active site cleft. Because the loop is already in an extended conformation, it cannot move away from the active site without unraveling at least one of the secondary structures (helix α6 and strand β3) to which it is attached. Interestingly, UCH-L3 has been crystallized in a configuration where the regions equivalent to loop L8 and helix α6 of UCH-L1 are disordered (9) as well as in conditions where these structures are ordered (24). This result suggests that at least helix α6 has the potential to exist in two different conformations, an ordered helical structure and a more flexible loop. If loop L8 is “designed”, to open and close on a regular basis, one would expect to find special features on the hinge regions (areas flanking the loop). Indeed, as shown in Fig. 1, the residues flanking loop L8, especially the side that is connected to helix α6 (right hinge), are conserved and are charged or polar in nature. In fact, the moiety that the right hinge rests on is also conserved with the same types of chemical properties, complementing each other like a piece of Velcro. Altogether, this analysis suggests that helix α6 can unravel, allowing loop L8 to move away from the active-site cleft. Interestingly, a similar dramatic unraveling of secondary structures and loop movement has been observed for protein kinases (26).

A Model for Ligand-Induced Conformational Change and Activation of UCH-L1. So far the structure of UCH-L1 implies that it is in an inactive state when free of ligand, that the secondary structures in the active site can move to form a productive triad, and that helix α6 can unravel to allow loop L8 to move away from the active-site cleft, thereby exposing the active site to its substrates. The question that remains is as follows: What can cause all these structural changes simultaneously? Binding of ubiquitin is not expected to make any structural changes to the P site, and ubiquitin should make little or no contact with the moieties that need to move, based on structures of UCH-L3 (24) and our model of the UCH-L1 and ubiquitin complex.

To date, the P’ ligand part of UCH-L1’s substrate is unknown, but if currently known substrates for other ubiquitin hydrolases are any indication, the substrate of UCH-L1 is likely to be a protein that is attached to the C terminus of ubiquitin via a surface lysine residue. In this scenario, as the substrate binds to UCH-L1 (P’ ligand to the P’ site and ubiquitin to the P site), loop L8 would push down and forward into the P’ site trough. This movement, in turn, would push helix α2 outward, breaking the H bonds between E60 and H161 and N159, releasing H161 along with strand β3 to move closer to C90, thereby forming an active enzyme–substrate complex. After hydrolysis and product release, L8 may spring back over the active-site cleft, serving as an on-off switch that regulates the function of UCH-L1.

Conclusions

The association of UCH-L1 with PD and cancer has generated great interest in understanding the biological role of UCH-L1 and its role in pathogenesis. Despite great efforts, these important questions remain elusive. A substitution mutation in UCH-L1 (I93M) was reported to be associated with familial early onset PD, whereas a different mutation (S18Y) was reported to be associated with reduced susceptibility to PD. The structure of UCH-L1 presented here shows that the side chain of I93 is in the hydrophobic core that holds the structure of the right lobe together (Fig. 8, which is published as supporting information on the PNAS web site). Both isoleucine and methionine are hydrophobic in nature; therefore, the side chain of methionine could exist in place of I93 without disrupting the hydrophobic core dramatically. Isoleucine, having a branched side chain, is more bulky than methionine, perhaps this difference in physical size may cause subtle perturbation in the immediate surroundings. Interestingly, I93 is conserved in UCH-L1 from all vertebrates except for Bufo gargarizans and Danio rerio, which contain valine, which also has a bulky side chain, at this position. The subtle perturbation caused by substituting methionine for isoleucine is unlikely to have significant structural consequences, but it may be significant for the function of UCH-L1 because I93 resides in helix α3, where the catalytic C90 is located, and any movement of this residue would distort the geometry of the catalytic triad. Such distortion may explain the observed reduced catalytic activity of the I93M mutant in vitro (14).

Residue S18 is in helix α1 and is solvent exposed. The type of amino acids at this position is variable among species, and there are no obvious structural consequences in the substitution of a tyrosine at the position of S18 in our structure. It is unclear, from a structural perspective, how the S18Y mutation affects the function of UCH-L1 that can cause a reduction in susceptibility to PD. Possibly this mutation may affect the interaction of this region of UCH-L1 with an as-yet-unidentified protein partner. Given that our structure of UCH-L1 indicates the protein exists in an inactive form on structures of UCH-L3 (24) and our model of the UCH-L1 and ubiquitin complex.

The identity of UCH-L1’s elusive substrate is vital in furthering our understanding of the biological role of UCH-L1. The structure...
presented in this report suggests that the activity of UCH-L1 is tightly regulated so as to be active only against a specific substrate(s), and that part of the substrate should possess a structure with a size and shape that complements the P-site presented in Fig. 3a. Because the expression of UCH-L1 is highly tissue-specific and restricted to cells with tight regulation of the cell cycle, it is possible that the substrate of UCH-L1 is a protein that is involved in such regulation.

**Experimental Procedures**

**Protein Expression, Purification, and Crystallization.** Full-length UCH-L1 was subcloned from a pcDNA-UCH-L1 vector into a pGEX-6P-1 vector (Amersham Pharmacia) by using standard cloning protocols. The resulting N-terminally fused glutathione S-transferase (GST)-tagged protein was expressed in *Escherichia coli* Rosetta strain (Novagen) and purified with a glutathione-Sepharose column (Amersham Pharmacia) according to the manufacturer’s protocols. The protein was further purified by size exclusion chromatography on a Superdex 75 column (Amersham Pharmacia). For crystallization, the protein solution (in 50 mM Tris HCl/150 mM NaCl, pH 7.4/10 mM DTT) was concentrated to 35 mg/mL. Crystals of UCH-L1 and its C220S variant were obtained from solution C4 of Hampton Research’s Grid Screen A/5 at ambient temperature.

**Crystalllographic Data Collection and Structure Determination.** Data (2.6 Å) from crystals of C220S mutant UCH-L1 were collected at beamline 23-ID of the Advanced Photon Source at Argonne National Laboratory. Diffraction data were collected at 100 K on a Mar225 CCD detector (Mar USA, Evanston, IL) and processed with the program HKL2000 (27). The crystals belonged to the space group *P*4_12_1 with two molecules per asymmetric unit. A homology model was constructed from the primary structure of UCH-L1 (National Center for Biotechnology Information Protein code NP_054472) and tertiary structure of UCH-L3 [Protein Data Bank (PDB) ID no. 1UCH] by using an online server SWISS-MODEL (28). The homology model was used as a molecular-replacement search model and yielded a solution with the program MOLREP (29). A 2F_F map following rigid body refinement showed reasonable density for ~70% of the model. Data of wild-type UCH-L1 crystals to 2.4-Å resolution were subsequently collected on beamline 9-1 at Stanford Synchrotron Radiation Laboratory. Diffraction data were collected at 100 K on a Q315 CCD detector (ADSC). Cycles of rebuilding and refinement were carried out by using the programs O (30) and CNS (31). The current R пт and R пт are 22.2% and 27.5%, respectively (rms deviations from ideal bond lengths and angles are 0.007 Å and 1.2°, respectively). The N-terminal five artificial residues were disordered and, therefore, excluded from the final model.

**Modeling and Graphics Presentation.** UCH-L1 was superimposed to UCH-L3-UbVMe (PDB ID code 1X03), UCH-L3 (PDB ID code 1UCH), Yuh1-Uthal (PDB ID code 1CMX) with the programs LSQKAB (32) of the CCP4 suite (33) and LSOMAN (34). Search for structures with similar fold was performed with the server DALI (35). Molecular graphical analysis was done with the programs O (30) and PYMOL (DeLano Scientific, South San Francisco, CA). Modeling and molecular simulation were performed with the program zNM (ZMM Software, Flamborough, ON, Canada). A homology model of UCH-L1 based on UCH-L3 (PDB ID code 1UCH) was created by the server SWISS-MODEL (28). Graphical analysis and displacement were done with the program PYMOL (DeLano Scientific).

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