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Alterations of structure and hydrolase activity of parkinsonism-associated human ubiquitin carboxyl-terminal hydrolase L1 variants

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Abstract

Ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) is a neuron-specific ubiquitin recycling enzyme. A mutation at residue 93 and polymorphism at residue 18 within human UCH-L1 are linked to familial Parkinson's disease and a decreased Parkinson's disease risk, respectively. Thus, we constructed recombinant human UCH-L1 variants and examined their structure (using circular dichroism) and hydrolase activities. We confirmed that an I93M substitution results in a decrease in k_{cat} (45.6%) coincident with an alteration in α-helical content. These changes may contribute to the pathogenesis of Parkinson's disease. In contrast, an S18Y substitution results in an increase in k_{cat} (112.6%) without altering the circular dichroistic spectrum. These data suggest that UCH-L1 hydrolase activity may be inversely correlated with Parkinson's disease risk and that the hydrolase activity is protective against the disease. Furthermore, we found that oxidation of UCH-L1 by 4-hydroxynonenal, a candidate for endogenous mediator of oxidative stress-induced neuronal cell death, results in a loss of hydrolase activity. Taken together, these results suggest that further studies of altered UCH-L1 hydrolase function may provide new insights into a possible common pathogenic mechanism between familial and sporadic Parkinson's disease.

Keywords: Ubiquitin; Proteasome; UCH-L1; Parkinsonism; Oxidative stress; 4-Hydroxynonenal

The ubiquitin–proteasome system is an evolutionarily conserved and energy-dependent proteolytic pathway. In addition to the elimination of misfolded and damaged toxic proteins produced in response to various cellular stresses, this pathway is responsible for turnover of various classes of short-lived proteins that control cell-cycle progression, specific gene transcription, membrane protein traffic, and intracellular signaling [1–4]. Ubiquitination of proteins is mediated by specific enzymes (E1, E2, and E3), and polyubiquitinated proteins are translocated to the proteasome and subsequently proteolytically degraded [3]. Conversely, deubiquitination is thought to be essential for the negative regulation of proteolysis and for recycling of ubiquitin from polyubiquitin chains [5].

Deubiquitinating enzymes consist of at least two families: the UBP (ubiquitin-specific processing protease) family [6–9] and the UCH family [7,8]. UBPs are thought to disassemble polyubiquitin chains whereas

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UCHs hydrolyze only small C-terminal ubiquitin adducts [8–10]. This restricted substrate specificity led to the proposal that UCHs function in ubiquitin recycling rather than in deubiquitination [11,12]. To date, four isoforms of UCH, L1–L4, have been identified in mammals [13–15].

Of the four UCH isoforms, UCH-L1 is neuron-specific [16,17]. Mutant mice with an intragenic deletion of the *UCH-L1* gene display neuron subtype-specific phenotypes and severe neurodegenerative disorders including gracile axonal dystrophy [18]. Previous studies of *UCH-L1*-associated human neurodegenerative diseases revealed single amino acid changes within UCH-L1 in some patients with Parkinson's disease (PD). An I93M missense mutation was identified in a German family affected by PD, and the partial loss of UCH-L1 hydrolase activity in this mutant may contribute to the disease [19]. Furthermore, an S18Y polymorphism encoded in exon 3 of human *UCH-L1* may be associated with decreased risk of PD in Caucasian, German, and Japanese populations [20–24] although no such association has been identified in other populations [25–28]. Recently, a novel enzymatic activity of UCH-L1 was discovered that affects the degradation of α-synuclein, a core component of Lewy bodies. The study demonstrated that the UCH-L1 dimer exhibits an ATP-independent ubiquitin ligase activity that inhibits the degradation of ubiquitinated α-synuclein (ubiquitinated at Lys 63) [29]. Furthermore, S18Y UCH-L1 ligase activity is lower than that of the wild-type enzyme, suggesting a possible link between the ligase activity and lowered risk of PD [29].

Although the I93M mutation and S18Y polymorphism of UCH-L1 could affect a single factor in the etiology of PD, common functional changes between the I93M and S18Y mutants of UCH-L1 have not been reported. In the present study, we reveal that UCH-L1 hydrolase activity is altered in both the I93M and S18Y UCH-L1 variants, suggesting that the pathogenesis of PD in both cases may have a common origin. We further demonstrate that UCH-L1 hydrolase activity is decreased by treatment with 4-hydroxynonenal (HNE), a candidate for endogenous mediator of oxidative stress-induced neuronal cell death, which is associated with sporadic PD. Thus, the hydrolase activity may also be involved in the pathogenesis of sporadic PD. Moreover, we also reveal that I93M UCH-L1 exhibits altered α-helical content relative to wild-type UCH-L1.

Materials and methods

Cloning of human UCH-L1. Human *UCH-L1* cDNA (GenBank Accession No. BC006305) was amplified by PCR using a human brain cDNA pool (Stratagene, La Jolla, CA) as template with the following primer sets: forward primer, 5′-GGGGCTCGAGCCGCGAAGAT GCAGCTCAAGCCGATGGAGATCAACCCCGAGATGCTGA-3′ (5′-GGGG-*Xho*I-CCGCGAAG-Met[1]-Gln-Leu-Lys-Pro-Met-Glu-Ile-Asn-Pro-Glu-Met-Leu[13]-3′), and reverse primer, 5′-GGGGGCGG CCGCTTAGGCTGCCTTGCAGAGAGCCA-3′ (3′-Ala[226]-Leu-Cys-Lys-Ala-Ala[233]-stop-*Not*I-GGGG-5′). Following an initial 3 min denaturation at 95 °C, the sample was amplified by 30 cycles of denaturation at 95 °C for 10 s, annealing at 53 °C for 20 s, and extension at 72 °C for 30 s. The amplified fragment was digested with *Xho*I and *Not*I and subcloned into the *Xho*I and *Not*I sites of pCI-neo (Promega, Madison, WI). Accuracy of the inserted nucleotide sequence was confirmed by sequence analysis.

Construction of bacterial expression plasmids for human UCH-L1 variants. Mutant cDNAs encoding human UCH-L1 containing either the I93M, S18Y, or C90S substitution were obtained using the QuikChange site-directed mutagenesis kit (Stratagene) with the following mutagenesis oligonucleotides: 5′-GAATTCCTGTGGCACAATG GGACTTATTCACGCAG-3′ and 5′-CTGCGTGAATAAGTCCC ATTGTGCCACAGGAATTC-3′ for I93M; 5′-GAACAAAGTGC TGTCCCGGCTGGGGGTCGC-3′ and 5′-GCGACCCCCAGCCGG GACAGCACTTTGTTC-3′ for S18Y; and 5′-CCATTGGGAAT TCCTCTGGCACAATCGGAC-3′ and 5′-GTCCGATTGTGCCA CAGGAATTCCCAATGG-3′ for C90S. Each single-nucleotide mutation in the resulting plasmids was confirmed by sequencing. Bacterial expression plasmids containing either 6HN-tagged human UCH-L1, I93M, S18Y, or C90S were constructed using a tetracycline-inducible expression system. *Xho*I–*Not*I cDNA fragments of the pCI-neo human UCH-L1 and mutant were digested, and then the DNA fragments were ligated between the *Sal*I and *Not*I sites in pPROtetE233 (Clontech, Palo Alto, CA) to generate pPROtetE233 6HN-tagged human UCH-L1, I93M, S18Y, and C90S vectors. The veracity of these expression plasmids was confirmed by sequencing.

Preparation of recombinant proteins. The pPROtetE233 6HN-tagged human UCH-L1, I93M, S18Y, and C90S vectors were transformed into *Escherichia coli* DH5αPRO. Purification of the recombinant proteins was performed using Co^{2+}–Sepharose (TALON purification kit, Clontech) according to the manufacturer's instructions.

Protein concentrations were determined using the BCA protein assay reagent (Pierce). Purified proteins were resolved by SDS–PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) under reducing conditions and visualized by Coomassie brilliant blue R-250 to confirm purity.

In vitro assay for human UCH-L1 activity. The hydrolase assay was performed as described in [30] with slight modifications. The assay for human UCH-L1 was carried out using a 96-well black assay plate at room temperature. Purified enzymes and the fluorogenic substrate ubiquitin-7-amino-4-methylcoumarin (Ub-AMC) (Boston Biochem, Cambridge, MA) were used to determine steady state kinetic parameters. The final enzyme concentration was 4.3 nM for UCH-L1, I93M, and S18Y mutants, and 5 μM for C90S. Before adding the substrate, the enzyme was incubated in assay buffer (20 mM Hepes, 0.5 mM EDTA, pH 7.8, containing 0.1 mg/ml ovalbumin, and 5 mM dithiothreitol) for 2 h to prevent oxidation of thiol groups. The substrate was diluted with assay buffer to a final concentration of 17.6–900 nM and a 50-μl aliquot was added to each well on the 96-well plate. The assay was initiated by the addition of 50 μl of stock enzyme solution. Fluorescence ($\lambda_{ex} = 355$ nm, $\lambda_{em} = 460$ nm) of AMC was monitored continuously using a Wallac 1420 multi-label counter (Perkin–Elmer, Turk, Finland), and the concentration of AMC was determined using standard curves of fully digested substrates. Initial velocity data were used to determine K_m and k_{cat} values from nonlinear fits of the Michaelis–Menten equation.

Circular dichroism. Circular dichroism (CD) measurements were performed as described in [31]. Purified recombinant human UCH-L1 and mutants were adjusted to a concentration of 0.1 mg/ml and dialyzed against 20 mM sodium phosphate buffer (pH 8.0). Far UV CD spectra (195–250 nm) were recorded in a 1-mm quartz cuvette on a Jasco J-820 spectropolarimeter (Jasco, Tokyo, Japan) equipped with a temperature controller by scanning at the rate of 50 nm/min at 20 °C. For all spectra, six scans were averaged. All CD spectra were corrected by background

subtraction for the spectrum obtained with buffer alone and smoothed. The observed ellipticity was normalized to units of degrees-cm^2/dmol. The spectra were analyzed for percent secondary structural elements by a computer program based on an algorithm that compares experimental spectra with those of known proteins [32]. Student's *t* test was used to compare the significance of the differences between data.

Modification of human UCH-L1. Recombinant 6HN-tagged human UCH-L1 proteins (0.3 mg/ml) were incubated with various concentrations (0–1.0 mM) of HNE (Calbiochem, San Diego, CA) in 60 μl of 50 mM sodium phosphate buffer (pH 7.2) for 2 h at 37 °C, as described in [33]. HNE-modification of UCH-L1 was monitored by SDS–PAGE and immunoblotting. Modified proteins (500 ng) were dissolved in the SDS–PAGE sample buffer and separated by 10–20% gradient SDS–PAGE prior to transfer to a PVDF membrane (Bio-Rad, Hercules, CA). After blocking with 5% nonfat dried milk and 3% goat serum in PBS, blots were probed with rabbit anti-HNE Michael Adducts antibody (1:2000, Calbiochem). Following washes, proteins reacting with a secondary antibody, HRP-conjugated goat anti-rabbit IgG (1:2000, Dako), were visualized using SuperSignal West Femto Maximum Sensitivity Substrate (Pierce).

Assay of residual hydrolase activity. HNE modified sample (55 μl) was applied to a MicroSpin G-25 Column (Amersham Pharmacia Biotech) to separate protein-bound HNE from free HNE. Recovery of the protein was established by SDS–PAGE and staining with Coomassie brilliant blue R-250. Residual activity of the modified enzyme was determined as described above. Initial velocity data were used to determine the values for relative activity.

Results

Purification and characterization of recombinant human UCH-L1 proteins

To obtain wild-type UCH-L1 and the variants I93M and S18Y associated with Parkinson's disease (Fig. 1A), N-terminal 6HN-tagged recombinant proteins were expressed in *E. coli* and purified by metal chelate affinity

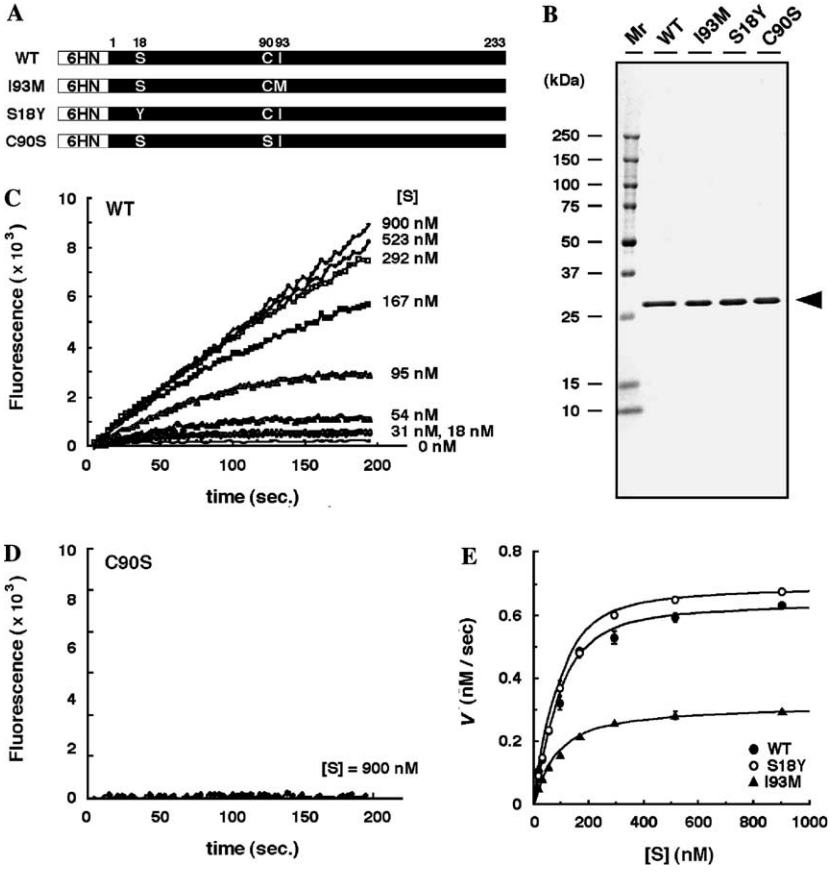

Fig. 1. Characterization and analysis of purified recombinant 6HN-tagged human UCH-L1s. (A) Schematic representation of 6HN-tagged human UCH-L1 wild-type (WT) and mutants I93M, S18Y, and C90S. The numbers indicate the amino acid residues of the N- and C-termini of UCH-L1 (open reading frame). The positions of the point mutations are indicated. The N-terminal 6HN-tag is shown in white. (B) Visualization of recombinant 6HN-tagged human UCH-L1s by SDS–PAGE under reducing conditions and Coomassie staining. One microgram of each sample was subjected to analysis. The *arrow* indicates the 28.9-kDa 6HN-tagged human UCH-L1 bands. M_r, molecular weight markers (kDa). (C) Kinetics of WT UCH-L1-catalyzed hydrolysis of Ub-AMC. Fluorescence intensity ($\lambda_{ex} = 355$ nm, $\lambda_{em} = 460$ nm) is indicated as a function of time. Enzyme concentration was 4.3 nM and substrate concentrations are indicated on the right. (D) Kinetics of C90S UCH-L1-catalyzed hydrolysis of Ub-AMC. Enzyme concentration was 4.3 μM and substrate concentration was 900 nM. (E) Effect of Ub-AMC concentration on the initial velocity of hydrolysis for WT UCH-L1 and variants. Activity measurements were performed using 4.3 nM 6HN-tagged human UCH-L1 or variant. Closed circle, WT; open circle, S18Y; closed triangle, I93M. Each value represents the mean ± SE of three independent experiments. Error bars are not shown if the error is smaller than the symbol.

chromatography. Coomassie staining of the purified proteins following SDS–PAGE showed a single 28.9-kDa band for each of the 6HN-tagged proteins (Fig. 1B) in good agreement with the theoretical 27.8-kDa molecular mass of the 6HN-tagged UCH-L1. The expression levels for the wild-type and variant UCH-L1 proteins were equivalent. As a negative control, we prepared a recombinant human C90S UCH-L1 in which serine is substituted within the active center triad [11] (Fig. 1A). The purified C90S UCH-L1 exhibited no hydrolase activity even at a very high concentration of 5 μM (Table 1; Fig. 1D), supporting the SDS–PAGE result (Fig. 1B) that there were no contaminant proteins with hydrolase activity in our recombinant protein preparations.

Kinetics of Ub-AMC hydrolysis by wild-type and variant UCH-L1s

UCH-L1 is a ubiquitin hydrolase that specifically cleaves small adducts of C-terminally modified ubiquitin (ubiquitin$^{1-72}$-Leu^{73}-Arg^{74}-Gly^{75}-Gly^{76}-X, where X can be any leaving group such as an amine, thiol group, small peptide, and polypeptide) [12]. To determine the enzymatic activities of recombinant human UCH-L1 and variants, we performed a general deubiquitinating assay using Ub-AMC as a substrate. Ub-AMC is efficiently hydrolyzed by UCH-L3 to liberate the highly fluorescent AMC moiety [30]. The hydrolase activity of wild-type UCH-L1 was compared with those of the parkinsonism-associated variants. Using varying concentrations of Ub-AMC, a Michaelis–Menten saturation curve was generated to determine the kinetics of catalysis for wild-type human UCH-L1 (Fig. 1C). Catalysis was saturated at 600–900 nM Ub-AMC (Figs. 1C and E). Next, steady-state kinetic parameters for each UCH-L1 mutant were determined using 4.3 nM wild-type and variant UCH-L1 and varying concentrations of Ub-AMC. The initial velocities were fit to the Michaelis–Menten equation to provide nonlinear least-squares parameters (Fig. 1E; Table 1). The k_{cat} for the wild-type UCH-L1 was 0.174 ± 0.0057 s^{-1}, while k_{cat} for the I93M and S18Y variants was 0.0794 ± 0.0034 s^{-1} and 0.196 ± 0.0028 s^{-1}, respectively (wild-type vs I93M, $p < 0.01$; wild-type vs S18Y, $p < 0.05$). The hydrolase activity of the I93M mutant was 45.6% of that of the wild-type UCH-L1. In contrast, the activity of the S18Y UCH-L1 variant was 1.13-fold higher than that of wild-type. The mutant and wild-type proteins exhibited similar K_m values, $K_m^{wt} = (122 \pm 10) \times 10^{-9}$ M, $K_m^{I93M} = (110 \pm 14) \times 10^{-9}$ M, and $K_m^{S18Y} = (136 \pm 1.7) \times 10^{-9}$ M, indicating that the mutant proteins showed similar affinity for Ub-AMC. In another study using recombinant UCH-L1, Liu et al. [29] reported that the S18Y and wild-type proteins exhibit equivalent hydrolase activities. The disagreement between their results and ours may be due to differences in protein preparations and/or hydrolase assay methods. We confirmed that the difference in the hydrolase activities of wild-type and S18Y UCH-L1s was statistically significant using independent protein preparations, and therefore we are confident that the hydrolase activity of S18Y UCH-L1 is higher than that of wild-type UCH-L1. The increased hydrolase activity appears to be a direct result of the S18Y mutation since the S18Y and wild-type UCH-L1 preparations contain no contaminant proteins or activities (Figs. 1B–D). Also, both proteins exhibit the same CD spectra (Fig. 2A). Significantly, the increased hydrolase activity of S18Y UCH-L1 was recently confirmed by another group [34].

Table 1
Kinetic parameters for hydrolysis of Ub-AMC by human UCH-L1s

Enzyme	k_{cat} (s^{-1})	K_m (nM)
UCH-L1 (wild-type)	0.174 ± 0.0057	122 ± 10
I93M	$0.0794^{**} \pm 0.0034$	110 ± 14
S18Y	$0.196^{*} \pm 0.0028$	136 ± 1.7
C90S[a]	N.D.[b]	N.D.[b]

Each value represents the means ± SE from three independent sets of experiments. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with values for UCH-L1 (wild-type) by t test.

[a] No hydrolyzed products were observed after 1 h with 5 μM enzyme.

[b] N.D., not detectable.

CD analysis of secondary structure in wild-type and variant human UCH-L1s

To address whether the observed differences in hydrolase activities of the variant UCH-L1s reflect altered secondary structure, we utilized CD spectroscopy to estimate secondary structure in the recombinant proteins (Fig. 2A). Human UCH-L1 and the variant proteins exhibited spectra with distinct minima at 208 and 222 nm, characteristic of high α-helical content (Fig. 2A). The ratios of α-helix, β-sheet, and other secondary structural features in these proteins were estimated from mean residue ellipticity data and are presented graphically in Fig. 2B. Relative to wild-type, the I93M mutant displayed slightly lower ellipticity over the range 195–200 nm, indicating decreased α-helical content (Fig. 2A, left). As a control, we prepared a recombinant double mutant UCH-L1 containing both the S18Y and I93M substitutions. The CD spectrum of the S18Y–I93M double mutant over 195–200 nm was similar to that of I93M (Fig. 2A, right), indicating that the amino acid substitution at position 93 is responsible for the decreased α-helical content. In contrast, the CD spectrum of the S18Y variant exhibited no difference compared to that of the wild-type protein.

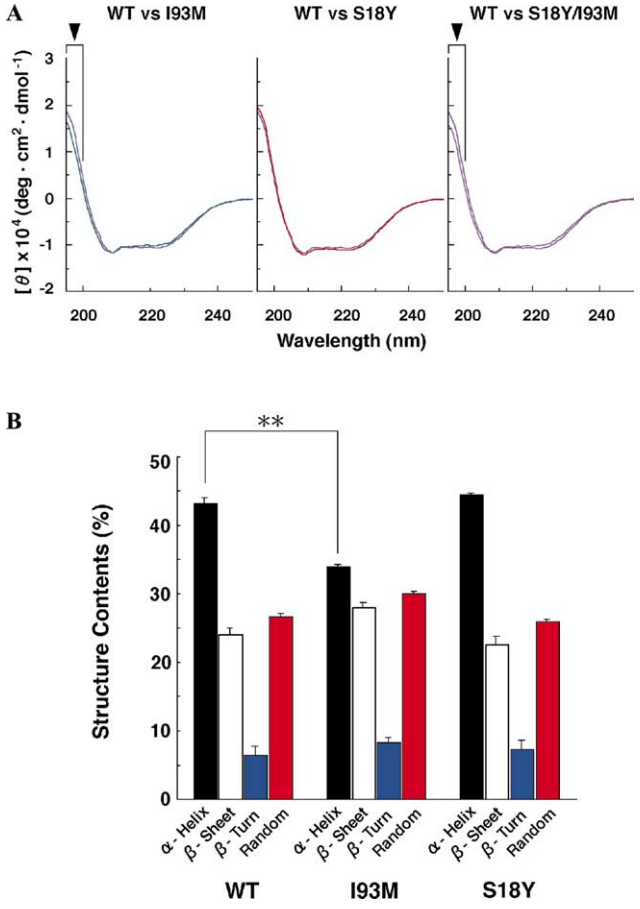

Fig. 2. CD spectra and secondary structural content of 6HN-tagged human UCH-L1s. (A) CD spectra (mean residue ellipticity) for recombinant proteins (0.1 mg/ml) in 50 mM sodium phosphate buffer. Wild-type UCH-L1 (WT) is shown in blue, I93M in green, S18Y in red, and S18Y-I93M in violet. Six scans were averaged for all spectra. *Arrowheads* indicate the differences of CD spectra. (B) Secondary structural content of recombinant human 6HN-tagged UCH-L1s. Each value represents the mean ± SE of six data sets. *p* value (*t* test) for the WT vs other proteins: **, < 0.01.

Modification and inactivation of UCH-L1 hydrolase activity by HNE

UCH-L1 belongs to a papain-like cysteine protease family [4,35] with conserved Cys and His residues within the active site. Certain cysteine proteases are known to be targets of cellular oxidative stress [36], and several lines of evidence implicate this phenomenon in sporadic PD [46]. One of the endogenous factors that is toxic to neurons during oxidative stress is HNE, an aldehyde product of fatty acid peroxidation. HNE can induce neuronal death [37,38] and is thought to form covalent cross-links with proteins via Michael addition to Cys, His, and Lys residues, thus altering the function of cysteine proteases [36]. HNE-modified proteins have been detected in nigral neurons and Lewy bodies in sporadic PD [39,40]. We examined whether HNE directly modifies/inactivates UCH-L1 in vitro. Past studies determined that HNE is produced in micromolar concentrations in response to certain oxidative stresses [41–43]. We utilized micromolar concentrations of HNE and 10 μM UCH-L1 for our experiments (UCH-L1 is an abundant protein, comprising 1–2% of soluble brain protein) [17,44]. Physiological concentrations of HNE (10–100 μM) were sufficient to covalently modify UCH-L1 and reduce hydrolase activity by 40–80% (Figs. 3A and B). Covalent modification also occurred at a lower

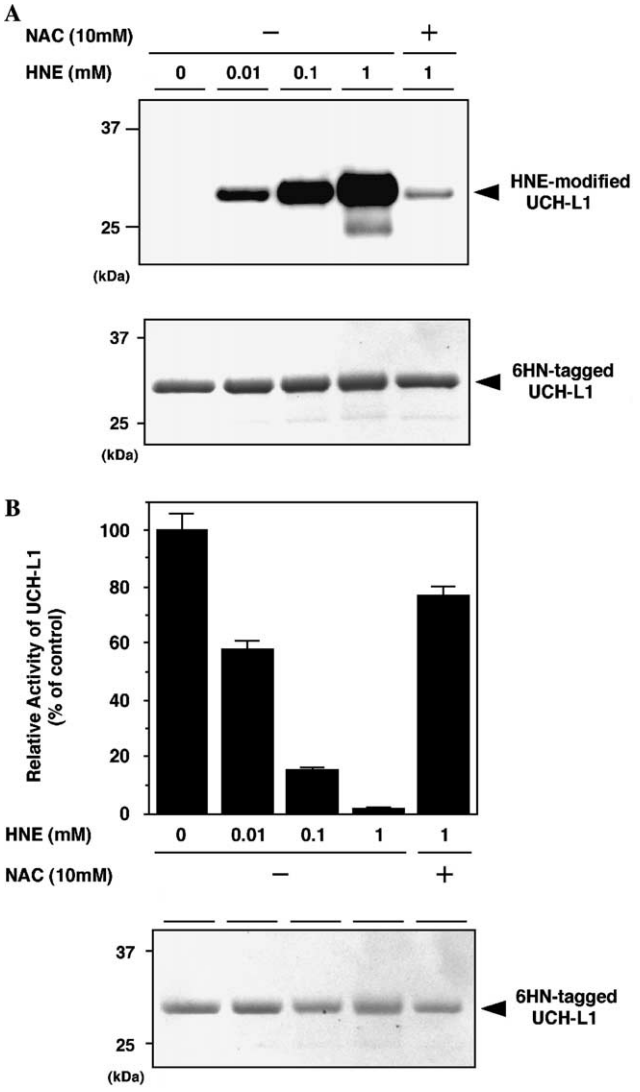

Fig. 3. Effect of HNE and NAC on UCH-L1 hydrolase activity. (A) Modification of wild-type UCH-L1 with HNE was visualized by immunoblot analysis with HNE adduct-specific antibody (top panel). HNE-modified UCH-L1 from the same sample was resolved by SDS–PAGE and stained with Coomassie brilliant blue (bottom panel). (B) Inactivation of UCH-L1 hydrolase activity by HNE and rescue with NAC (top panel). The residual activities of HNE-modified UCH-L1 prepared from the same sample shown in (A) were measured by hydrolase assay. Each value represents the mean ± SE of four experiments. The HNE-modified UCH-L1 proteins were separated from free HNE, resolved by SDS–PAGE, and stained with Coomassie brilliant blue (bottom panel).

concentration of UCH-L1 (1.25 μM; data not shown). Moreover, excess *N*-acetyl-L-cysteine (NAC: as a competitive cysteine analogue) prevented both HNE modification (Fig. 3A) and the decrease in hydrolase activity, thus confirming that human UCH-L1 was modified with HNE (Fig. 3B).

Discussion

We analyzed the hydrolase activities of parkinsonism-associated UCH-L1 variants, I93M and S18Y. The I93M mutation causes a decrease in the hydrolase activity in agreement with a previous report [19]. Furthermore, the S18Y variant exhibits slightly higher hydrolase activity than the wild-type enzyme. We also examined the structural features of the UCH-L1 variants by CD spectroscopy. The wild-type and S18Y UCH-L1 proteins elicit essentially identical CD spectra while the I93M mutant exhibits a decrease in α-helical content. Finally, we found that UCH-L1 modification by HNE decreases its hydrolase activity.

The I93M UCH-L1 mutant exhibits reduced hydrolase activity (~55% of wild-type), and this mutant displays 80% penetrance in a German family carrying the allele [19]. Conversely, genetic-epidemiological studies demonstrated that carriers of the S18Y UCH-L1 polymorphism that causes increased hydrolase activity show a decreased risk of sporadic PD. Furthermore, the risk of PD is also dependent on the S18Y allele dosage in that homozygotes of this allele are at lower relative risk (0.31) than heterozygotes (relative risk between 0.55 and 0.81) [21,24,25]. Thus, the PD risk appears to correlate inversely with the hydrolase activity of the UCH-L1 variants associated with PD (Table 2), suggesting that increased hydrolase activity may confer protection against the development of PD while decreased activity may be involved in PD pathogenesis.

Another study using recombinant UCH-L1 proteins demonstrated that UCH-L1 has dual enzymatic activity, both as a ubiquitin hydrolase and an ATP-independent ubiquitin ligase for α-synuclein [29]. It was also shown that the ligase activity of S18Y UCH-L1 is decreased relative to that of the wild-type UCH-L1. These results suggest that the ligase activity, in addition to the hydrolase activity, is involved in PD etiology. Our results demonstrate that the I93M mutation both impairs hydrolase activity and perturbs the structure in the protein. Given that UCH-L1 is an abundant protein (1–2% of soluble brain protein) [17] and constitutes a principal component of Lewy bodies in sporadic PD [45], such a structural alteration may contribute to the accumulation/aggregation of UCH-L1 (and/or mutants thereof) in the diseased state. Thus, there may be a correlation between structural changes within UCH-L1 and PD-associated Lewy body formation. However, further detailed biochemical and biophysical characterization of the I93M UCH-L1 mutant is required to confirm this possibility.

The protein structure is altered only in I93M UCH-L1 and the ligase activity is decreased both in I93M and S18Y UCH-L1s [29]. However, only the hydrolase activity correlates inversely with the risk of PD (Table 2). Therefore, the change in hydrolase activity in both I93M and S18Y UCH-L1 variants may be responsible, in whole or part, for the differences in PD risk observed in the two populations carrying these distinct alleles.

Two other causative genes, *parkin* and *α-synuclein*, have been linked to familial PD (reviewed in 46). Parkin exhibits E3 ubiquitin ligase activity [47,48]. Parkin-associated endothelin receptor-like receptor (Pael-R) has been identified as a substrate for parkin [49]. Parkin is thought to remove misfolded proteins such as Pael-R from the endoplasmic reticulum (ER) and to protect neurons from ER-mediated stress-induced cell death [49]. α-Synuclein, a core component of Lewy bodies [50], is also a substrate for parkin and therefore parkin may be involved in Lewy body formation as well [51]. It was proposed that UCH-L1 is a neuron-specific ubiquitin hydrolase required to maintain cellular levels of 'free ubiquitin' (i.e., ligatable at the C-terminus) [12]. The decreased hydrolase activity of the I93M UCH-L1 mutant possibly results in reduced levels of free ubiquitin that may adversely affect the normal degradation of Pael-R and α-synuclein. Conversely, the increased hydrolase activity of the S18Y mutant may elevate cellular free ubiquitin levels, resulting in enhanced degradation of Pael-R and α-synuclein. Moreover, the increased hydrolase activity of S18Y UCH-L1 coupled with its decreased ligase activity (which can prevent α-synuclein degradation by K63-linked ubiquitin ligation) [29] may be additive or synergistic with respect to the decreased risk of sporadic PD. These hypotheses may be confirmed in future in vivo studies designed to measure cellular

Table 2
Association between Parkinson's disease and human UCH-L1 mutants

UCH-L1 variant	WT	I93M	S18Y	References
Incidence of Parkinson's disease		↑	↓	[19–24]
Hydrolase activity	(100%)	↓ (45.6%)	↑ (112.6%)	[19, this study]
CD spectra alteration	Normal	↓ (α-Helix)	Normal	
Ubiquitin ligase activity	(100%)	↓	↓↓	[29]

levels of C-terminal-modified and -free ubiquitin in nigral neurons expressing PD-associated UCH-L1 variants.

Oxidative stress is believed to play a role in the pathogenesis of sporadic PD by promoting the generation and accumulation of oxidant-modified proteins that exhibit aberrant properties [46,52]. HNE, a good candidate for an endogenous toxic oxidizing factor, possibly affects the neuronal ubiquitin–proteasome pathway since UCH-L1 is directly modified by HNE in vitro, resulting in a significant decrease in hydrolase activity. This loss of activity could affect the level of free ubiquitin in a manner similar to that of the UCH-L1 mutants, and consequently may affect the risk of PD. Therefore, the disruption of control mechanisms within the ubiquitin–proteasome pathway via changes in UCH-L1 hydrolase activity may represent a common origin for the pathogenesis of both sporadic and familial PD.

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