Bioorganic & Medicinal Chemistry Letters 22 (2012) 3900–3904

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

The co-crystal structure of ubiquitin carboxy-terminal hydrolase L1 (UCHL1) with a tripeptide fluoromethyl ketone (Z-VAE(OMe)-FMK)

Christopher W. Davies [a], Joseph Chaney [a], Gregory Korbel [b], Dagmar Ringe [c], Gregory A. Petsko [c], Hidde Ploegh [b], Chittaranjan Das [a,*]

[a] Department of Chemistry, Purdue University, West Lafayette, IN, USA
[b] Whitehead Institute for Biomedical Research, Cambridge, MA, USA
[c] Rosenstiel Basic Medical Sciences Research Center, 415 South Street, MS-029, Waltham, MA, USA

ARTICLE INFO

Article history:
Received 8 February 2012
Revised 25 April 2012
Accepted 27 April 2012
Available online 4 May 2012

Keywords:
Ubiquitin hydrolase
Deubiquitinating enzymes
Fluoromethyl ketone
Enzyme inhibitors

ABSTRACT

UCHL1 is a 223 amino acid member of the UCH family of deubiquitinating enzymes (DUBs), found abundantly and exclusively expressed in neurons and the testis in normal tissues. Two naturally occurring variants of UCHL1 are directly involved in Parkinson's disease (PD). Not only has UCHL1 been linked to PD, but it has oncogenic properties, having been found abnormally expressed in lung, pancreatic, and colorectal cancers. Although inhibitors of UCHL1 have been described previously the co-crystal structure of the enzyme bound to any inhibitor has not been reported. Herein, we report the X-ray structure of UCHL1 co-crystallized with a peptide-based fluoromethylketone inhibitor, Z-VAE(OMe)-FMK (VAEFMK) at 2.35 Å resolution. The co-crystal structure reveals that the inhibitor binds in the active-site cleft, irreversibly modifying the active-site cysteine; however, the catalytic histidine is still misaligned as seen in the native structure, suggesting that the inhibitor binds to an inactive form of the enzyme. Our structure also reveals that the inhibitor approaches the active-site cleft from the opposite side of the crossover loop as compared to the direction of approach of ubiquitin's C-terminal tail, thereby occupying the P1′ (leaving group) site, a binding site perhaps used by the unknown C-terminal extension of ubiquitin in the actual in vivo substrate(s) of UCHL1. This structure provides a view of molecular contacts at the active-site cleft between the inhibitor and the enzyme as well as furnishing structural information needed to facilitate further design of inhibitors targeted to UCHL1 with high selectivity and potency.

UCHL1, a protein normally expressed exclusively in the brain and testis, is a member of the ubiquitin carboxy-terminal hydrolase (UCH) family of enzymes, a subclass of a larger group of enzymes collectively called deubiquitinases (DUBs).[1,2] DUBs catalyze the hydrolysis of isopeptide or peptide bonds between ubiquitin and target proteins, or between monomers in polymeric chains of ubiquitin.[3–6] Although the exact in vivo substrate(s) for this enzyme is not known, biochemical studies have shown that UCHL1 is active only towards ubiquitin conjugates with small leaving groups at the C-terminus of ubiquitin.[7,8] There are five classes of DUBs, four of which are cysteine proteases including the UCH family, the fifth class being zinc metalloproteases.[4–6,9] UCHL1, like its other family members, UCHL3, UCHL5, and BAP1, contains a catalytic triad consisting of a cysteine (Cys90), a histidine (His161), and an aspartate (Asp176). The X-ray crystal structure of the enzyme is known and reveals a misaligned catalytic triad with a Cys-His distance of ~8 Å, much further than a catalytically competent distance of ~4 Å for a cysteine protease.[10] However, upon binding to ubiquitin, as revealed in the co-crystal structure of this enzyme with the ubiquitin-based suicide substrate, ubiquitin vinylmethylester (UbVME), the catalytic triad adopts a productive arrangement as seen in other active cysteine proteases.[11]

The physiological function of UCHL1 is not known; however, abnormal expression of UCHL1 is observed in many forms of cancers, including colorectal, lung and pancreatic cancers.[12–14] UCHL1 transgenic mice are prone to malignancy, primarily lymphomas and lung tumors,[15] and the gad (gracile axonal dystrophy) mouse exhibits severe neurologic defects.[16] UCHL1's role in disease makes it a possible target for design of therapeutics in the form of small-molecule inhibitors. Many other cysteine proteases have been so targeted, because elevated and uncontrolled levels of protease

Abbreviations: Z-VAD-FMK, benzyloxycarbonyl-Val-Ala-Asp fluoromethylketone; Z-VAE(OMe)-FMK, benzyloxycarbonyl-Val-Ala-Glu(γ-methoxy) fluoromethylketone; BAP1, BRCA associated protein-1; CMK, chloromethyl ketones; DUBs, deubiquitinases; FMKs, fluoromethylketones; gad, gracile axonal dystrophy; MR, molecular replacement; UCHL1, ubiquitin carboxy-terminal hydrolase L1; UCHL3, ubiquitin carboxy-terminal hydrolase L3; UCHL5, ubiquitin carboxy-terminal hydrolase L5; UbVME, ubiquitin vinylmethyl ester.

* Corresponding author. Address: Department of Chemistry, Purdue University, Brown Laboratory of Chemistry, 560 Oval Drive, West Lafayette, IN 47907, USA. Tel.: +1 765 494 5478; fax: +1 765 494 0239.
E-mail address: cdas@purdue.edu (C. Das).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.04.124

activity can result in physiological imbalance, leading to the onset of several diseases.[17,18] One of the most studied cysteine protease targets for inhibition is the caspase family of cysteine proteases, the members of which are covalently inactivated by peptide halomethyl ketones such as Z-VAD-FMK (benzyloxycarbonyl-Val-Ala-Asp fluoromethylketone).[19] FMKs (fluoromethylketones) are irreversible inhibitors of cysteine proteases by virtue of their ability to alkylate the active-site thiol, leading to the displacement of the halide group by the catalytic cysteine to form a thioether bond between the cysteine and the inhibitor.[18,20]

Here we report the X-ray co-crystal structure of UCHL1 with a tripeptide fluoromethylketone, Z-VAE(OMe)-FMK (benzyloxycarbonyl-Val-Ala-Glu(γ-methoxy) fluoromethylketone),[21] at 2.35 Å resolution. The co-crystal structure reveals that the inhibitor binds in the active-site cleft, irreversibly modifying the active-site cysteine. However, the catalytic histidine is still misaligned as seen in the native structure,[10] suggesting that the inhibitor binds to an inactive form of the enzyme. Our structure also reveals that the inhibitor approaches the active-site cleft from the opposite side of the crossover loop as compared to the direction of approach of ubiquitin's C-terminal tail thereby occupying the P1′ site, a binding site perhaps used by the unknown C-terminal extension of ubiquitin in the actual in vivo substrate(s) of UCHL1. Our structure provides both the first view of molecular contacts at the active-site cleft between an inhibitor and this enzyme as well as the structural information needed to facilitate further design of inhibitors toward UCHL1 with high selectivity and potency.

Z-VAE(OMe)-FMK (hereafter referred to as simply VAEFMK) was synthesized as described previously.[21] We identified VAEFMK as an inhibitor of UCHL1 during a screen of inhibitors for the viral DUB, UL36 (of herpes simplex virus type 1). During that screen, which included several CMK (chloromethyl ketone) and FMK based putative cysteine protease inhibitors, it was observed that some of these molecules inhibited UL36 both in lysates and in cell culture (unpublished data). The assay employed here relied on the ability of the DUB to react with HA (hemagglutinin)-tagged ubiquitin vinylmethyl ester (HA-UbVME). Curiously, we found one particular compound, VAEFMK, effectively inhibited UCHL1's reaction with HA-UbVME at the concentration of 100 μM; however, there was no effect on UCHL3 and UCHL5 at that concentration. On the other hand, a related compound, Z-VAD-FMK, was unable to inhibit UCHL1 activity even at 440 μM concentration (Supplementary Fig. 2). VAEFMK was co-crystallized with wild-type UCHL1 (upon covalent modification of the enzyme's thiol, the fluoride group is lost (see mechanism below), therefore, the fluoride group is not seen in the structure and the actual adduct we crystallized should be referred to as UCHL1-VAE thioether). The co-crystal structure was solved by molecular replacement (MR) using the structure of UCHL1 (Protein Data Bank (PDB) code: 2ETL) as the search model. After MR, the electron density map ($2F_O$–F_C) was inspected for presence of positive density that could correspond to bound inhibitor. Inspection of the map indeed revealed positive density corresponding to the inhibitor within the active-site cleft. Using ProDrug Server,[22] we generated a model of the inhibitor and placed the molecule within the positive density and carried out rounds of restrained refinement combined with rounds of model building, which resulted in a final free R (R_{free}) and a crystallographic R (R_{crys}) of 25.0 and 19.7%, respectively (Table 1). The final refined model contains the complete 223 amino acids of the wild-type UCHL1 with a single molecule of bound inhibitor. The pentapetide fragment carried over from the GST (glutathione S-transferase) expression tag after cleavage by PreScission Protease (GE Biosciences, USA) was disordered and therefore not modeled. Similarly to the native UCHL1 crystal, the model contains a dimer in the asymmetric unit, with each monomer in the asymmetric unit containing one copy of the inhibitor.

Table 1
Crystallographic table of statistics

	UCHL1-VAE
Residues	1–223
Data collection	
Space group	$P\,42_12$
Cell dimensions	
a, b, c (Å)	110.0, 110.0, 78.7
α, β, γ (°)	90, 90, 90
Resolution (Å)	50.0–2.35 (2.43–2.35)
$^*R_{sym}$	5.1 (49.9)
I/σ I	48.5 (2.1)
Unique reflections	19259
Completeness (%)	98.1 (89.6)
Redundancy	12.9 (7.8)
Refinement	
Resolution	34.9–2.35 (2.43–2.35)
$^1R_{crys}/R_{free}$	19.7/25.0
Number of atoms	3589
DRG Molecules	2
rmsd	
Bond lengths (Å)	0.005
Bond angles (°)	1.0
Ramachandran plot	
Most favored (%)	91.4
Additionally allowed regions (%)	7.3
Generously allowed regions (%)	0.3
Disallowed (%)	1.0
Bfactors (Å²)	
Protein	76.7
Solvent	59.4
DRG	93.6

$^*R_{sym} = \Sigma_{j|} I(h)_j - \langle I(h)\rangle| / \Sigma_j I(h)_j$, where $I(h)_j$ is the scaled observed intensity of the jth observation of reflection h, and <I(h)>is the mean value of corresponding symmetry-related reflections.
$^1R_{crys} = \Sigma ||F_{obs}| - |F_{calc}|| / \Sigma |F_{obs}|$ and $R_{free} = \Sigma ||F_{obs}| - |F_{calc}| / \Sigma |F_{obs}|$, where R_{free} and R_{crys} are calculated using a randomly selected test set of 5% of the data and all reflections excluding the 5% test data, respectively. Numbers in parentheses are for the high-resolution shell.

VAEFMK is a peptide-based inhibitor that resembles the well-known caspase inhibitor Z-VAD-FMK (Fig. 1a). The usual mechanism of FMK inhibitors proceeds via a two-step addition followed by collapse/migration/displacement mechanism. Fluorine being a highly electron withdrawing group combined with the inductive effects of the carbonyl carbon oxygen makes the carbonyl carbon more electrophilic than the alpha carbon where the substitution ends up. Covalent modification proceeds by an initial nucleophilic attack on the carbonyl carbon by the reactive thiol from the enzyme, followed by the collapse of the carbonyl, migration of the thiol to the alpha carbon, and subsequent displacement of the

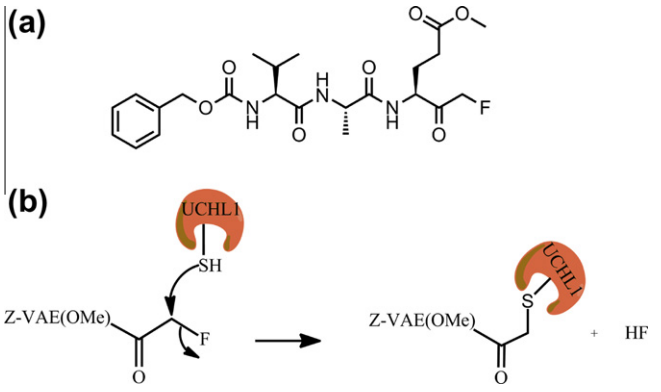

Figure 1. VAEFMK. (a) Structure of Z-VAE(OMe)-FMK (VAEFMK). (b) UCHL1 is irreversibly modified by VAEFMK.