Investigating the Binding Interactions between Inhibitors and Ubiquitin C-Terminal Hydrolases for Parkinson Disease Research

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Abstract

Ubiquitin C- terminal hydrolase (UCH-L1) is one of the most abundant proteins in the brain, constituting up to 2% of the total protein in the brain. UCH-L1 is normally expressed in neurons and testis. The expression of UCH-L1 was found to correlate with Parkinson disease and also tumor progression. Developing effective inhibitors to selectively inhibit the function of UCH-L1 can pave the path to understanding the molecular mechanism of Parkinson disease. From this research, a set of inhibitors and non-inhibitors screened from previous research was docked using molecular docking methods. It was revealed the specific active site of UCH-L1 the inhibitors bind to as well as an understanding of the type of inhibitors that will be effective. It was found that inhibitors that were docked yielded a higher binding energy than the non-inhibitors bound to the active site. The average binding energy for inhibitors were -7.91 kcal/mol and for non-inhibitors were -7.27 kcal/mol. When examining the geometry and interaction of inhibitors and non-inhibitors, it was determined that the biggest effect that contributes to a stronger binding affinity is π-π interaction and hydrophobic interaction.

Introduction

Proteins in eukaryotic cells can be covalently modified, usually only transiently, by certain other proteins. Among those protein modifiers, is ubiquitin. Ubiquitin (Ub) is a small protein that can be transiently attached to thousands of different proteins. The small 76-amino acid protein plays a critical role in the cellular processes by the attachment to other proteins that lead to proteasomal degradation. Ubiquitylation is also involved in nonproteolytic regulatory mechanism, such as membrane protein endocytosis and intracellular trafficking, chromatin-mediated regulation of transcription, DNA repair, and assembly of signaling complexes. The process also controls the sorting and localization of certain proteins in a reversible manner, such as phosphorylation modulates changes in the structure, activity and the localization of the target proteins. As such, deubiquitylating enzymes (DUBs) act analogously to phosphatases that function in phosphorylation processes.

DUBs subdivided into Ub- C-terminal hydrolases (UCHs) and Ub-specific proteases (UBPs). A member of the UCH family of DUBs is a 223- amino acid protein, UCHL1, found abundantly and selectively expressed in brain, constituting up to 1-2% of total brain protein. UCHL1 is a cysteine protease, with a catalytic triad consisting of cysteine (Cys90), histidine (His61), and aspartate (Asp176). Studies showed evident signs of diseases correlated with UCHL1 including tumor progression, severe forms of mental retardation such as Angelman's Syndrome, neurodegenerative disorder such as Parkinson's, Huntington's, and Alzheimer's disease, and diabetes. Recent studies showed a positive correlation existing between UCHL1 expression and tumor progression. The study focused on the relationship of UCHL1 and tumor progression as well as possible inhibitors that opposes proliferation. This class of inhibitors that was found was O-acyl oxime derivatives of isatins. The molecular mechanism of responses found in the study is still yet to be determined.

We chose to examine the molecular dynamics between such inhibitors and UCHL1 protein to get a better understanding of the alterations in functionality as well as the mechanism between ligand and protein.

Methods

The docking study was conducted using the online software SwissDock. SwissDock is a program that predicts the molecular interactions that may occur between a target protein and a small molecule. Within SwissDock, the docking tool EADock DSS is composed of algorithms consisting many binding modes that are generated either in a box (local docking) or in the vicinity of all target cavities (blind docking). The CHARMM energies are simultaneously estimated on a grid and the binding modes with the most favorable energies are evaluated with FACTS, and clusters. The protein files were obtained from the Protein Data Bank for UCHL1 protein (2ETL). The geometry of UCHL1 was frozen, and the ligand (inhibitor) atoms were allowed to be flexible within 5Å of a distance.

Inhibitors and Non-Inhibitors bound to UCH-L1

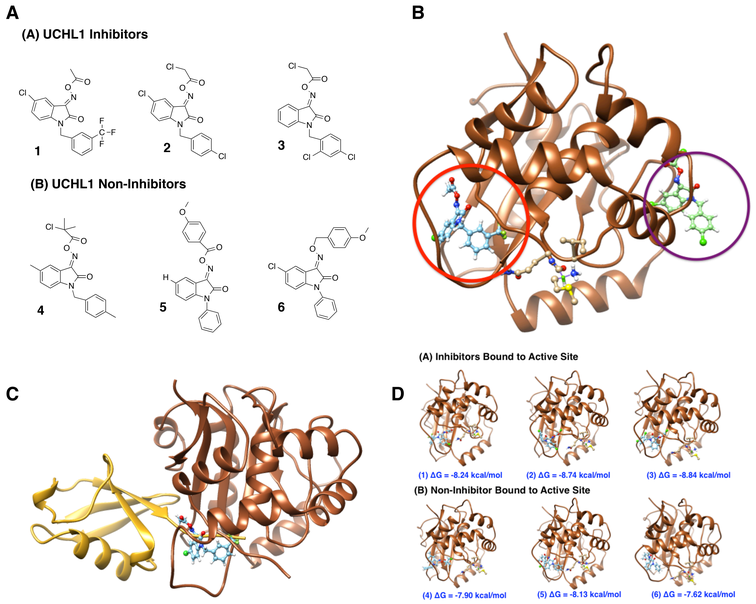

Figure 1. (A) Chemical structures of the inhibitors and non-inhibitors used to dock on UCH-L1 protein, (B) Two favorable binding sites found on UCH-L1 protein from the docking results shown in red and purple circles. The red circle was the site that was determined as an active binding site. (C) UCH-L1 protein (brown) bound to an ubiquitin (yellow) protein, and inhibitor bound to the active site. (D) The binding conformations of between the inhibitor (non-inhibitor bound) to the UCH-L1 protein. The label in blue is the binding energy (ΔG) in kcal/mol for each inhibitors and non-inhibitors.

Geometry and Interaction of Ligand and UCH-L1

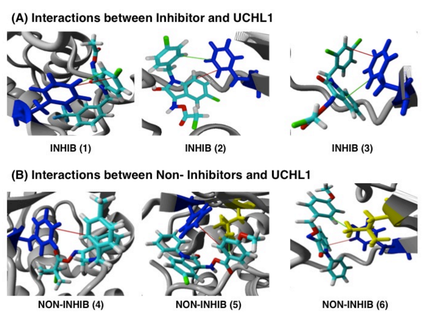

Figure 2. The binding interactions between UCH-L1 and ligands. The hydrophobic interactions are represented by green lines and π-π interactions are represented by a red line. For inhibitors, the hydrophobic interactions and and π-π interactions occur with phenylalanine (Phe) 160 represented in blue. For the last two non-inhibitors, the hydrophobic interaction occur with Leucine (Leu) 52 represented in yellow.

Proposed Inhibitors for UCH-L1

Proposed UCHL1 Inhibitors

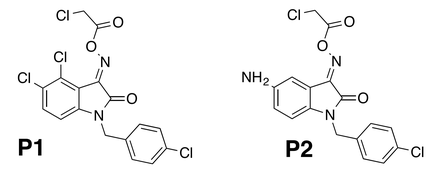

Figure 3. Proposed inhibitors that have exhibit improved binding energy with UCH-L1 protein. P1 is the proposed inhibitor with an average binding energy of -8.34 kcal/mol. P2 is the proposed inhibitor with an average binding energy of -8.04 kcal/mol.

Comparison of Binding Energy and VdW Energy

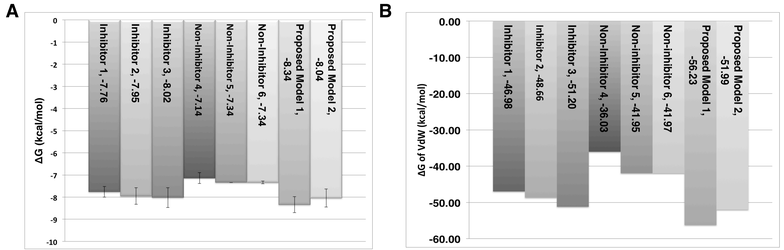

Figure 4. Comparison of binding energies (A) and van der Waals interactions (B) between inhibitor, non-inhibitor, and proposed inhibitors. In both graphs, the proposed inhibitor yielded a higher binding energy and VdW energy than the inhibitors in the study.

Conclusions

After the molecular docking of the inhibitors and non-inhibitors on UCH-L1 protein, the molecular interaction that labels compounds "inhibitors" and "non-inhibitors" were analyzed. The inhibitors in the study yielded a higher binding free energy than the non-inhibitors, which is consistent with the experimental findings in experiments.

When examining the components that creates such high binding free energy, it was determined that the π –π interactions and hydrophobic interactions play a crucial role. The inhibitors had a higher van der Waals interaction energy than the non-inhibitors. With this knowledge, two new inhibitors were proposed. To increase the VdW energy, electron donor groups were placed on the aromatic ring of the inhibitor. The docking results showed that the proposed inhibitors indeed exhibited a higher binding energy (as well as a higher van der Waals interaction energy as expected).

Our study on the interactions between UCH-L1 protein and inhibitor ligands provided the new insights at the molecular level for designing better inhibitors. Our proposed inhibitors will lead to new paths to explore the molecular origins of Parkinson diseases..