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

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Abstract
UCH-L1 is a 223-amino acid protein that is abundantly found in the brain. This protein has been linked to many neurodegenerative disorders, tumor progression, and mental retardation. A previous research was done that provided an insight to the inhibition of the UCH-L1 protein that prevents tumor progression. It was found that O-acyl oxime derivatives of isatin were effective inhibitors that allowed the cellular aggregation that led tumor progression. The molecular mechanism that led to such response was still yet to be determined. We used docking software, SwissDock, to understand the molecular mechanisms of the inhibitors that were found in the research. From the results, we determined that the inhibitors yielded a higher binding energy of -7.91 kcal/mol. The inhibitors that exhibited non-activity yielded a binding energy of -7.27 kcal/mol. When analyzing each structure of the inhibitors and non-activity inhibitors, it was found that most effective interaction that leads to a better binding energy is π-π interaction between the aromatic ring of isatin and phenylalanine (Phe) 150. It was noted that a hydrophobic interaction occurs as well for two of the non-activity inhibitors.

Introduction

Proteins 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 (1). 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 (1,3).

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 (4). UCHL1 is a cysteine protease, with a catalytic triad consisting of cysteine (Cys90), histidine (His61), and aspartate (Asp176) (4). 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 (1, 3). 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 (2).

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.

Methodology

Drawing of the Inhibitors and Non-Inhibitors
Three effective inhibitors and non-activity inhibitors of O-acyl oxime derivatives of isatin were chosen for docking (Fig. 1). The inhibitors and non-inhibitors that were used in this study were drawn using Avogadro. The ligands were drawn and equilibrated for proper geometric conformation.

Docking of the Inhibitors and Non-activity Inhibitors
The docking study was conducted using a powerful system called SwissDock. SwissDock is a program that predicts the molecular interactions that may occur between a target protein and a small molecule. The docking software 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 (5).

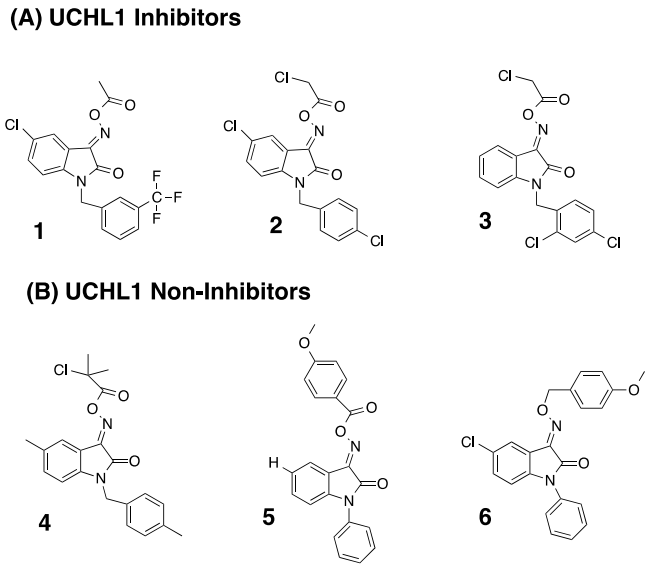

Figure 1. Illustrates the chemical make-up of the inhibitor and non-inhibitor of O-acyl oxime derivative of isatin used for docking. Compounds (1-3) show the UCHL-1 protein inhibitors and (4-6) show the UCH-L1 non-inhibitors.

Results

From the docking results, it was evident that both inhibitor and non-inhibitors favored two binding sites on the UCH-L1 protein. When the UCH-L1 protein was superposed on Ubiquitin protein, it was shown that the binding site labeled in red was the more favorable binding site (Figure 3).

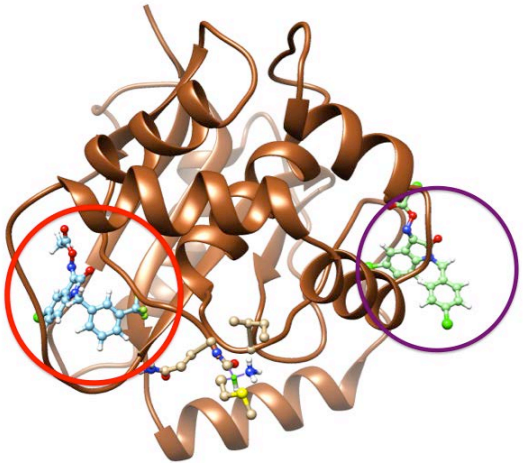

Figure 2. Two binding sites labeled in red and purple circle are shown on the UCH-L1 protein represented in brown. The inhibitors and non-inhibitors bound to either the pocket of the red circle or of the purple circle.

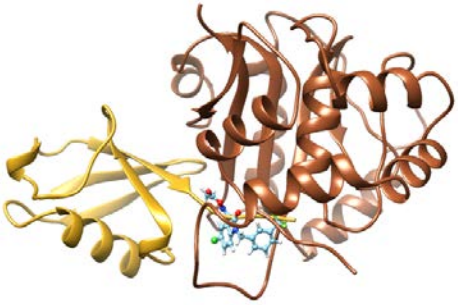

Figure 3. UCH-L1 protein was superposed onto an ubiquitin molecule represented in yellow. It was evident that the binding site labeled in red circle was the active binding site between the ubiquitin protein and UCH-L1 protein.

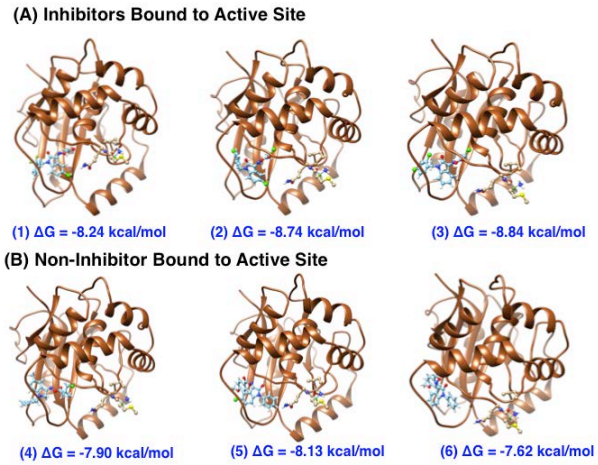

Figure 4. Illustrates the inhibitors and non-inhibitors bound to UCHL1 protein at the respected conformation that exhibit the highest binding energy. The label in blue tells the binding energy (ΔG) in kcal/mol for each inhibitors and non-inhibitors. The inhibitors bound to the active site exhibited a higher binding energy than the non-inhibitors.

It was calculated that the average binding energy of inhibitors were higher than non-inhibitors bound to the active site (Figure 5). When analyzing the geometry and interaction between inhibitors and non-inhibitors with UCHL1 protein, it was found that all inhibitors had hydrophobic and π-π interactions with phenylalanine (Phe 160) of the UCHL1 protein. While the non-inhibitors exhibited the same interaction as the inhibitors, the interaction was with other residues of the UCHL1 protein. For non-inhibitors 5 and 6, a hydrophobic interaction was found with Leucine (Leu52) and π-π interactions with phenylalanine (Phe 160) of the protein. Non-inhibitor 4 showed interactions of hydrophobic and π-π with Phe160 but the bonds intersect over another to different locations of the benzene ring on phenylalanine (Figure 6).

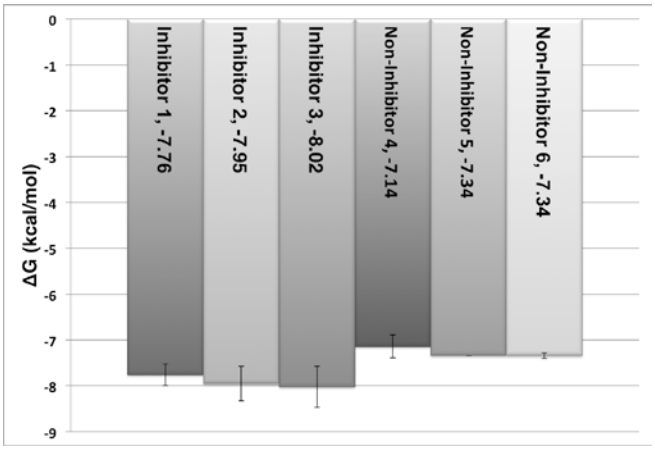

Figure 5. Illustrates the ΔG energy for each inhibitor and non-inhibitor bound to UCHL1 protein. The error bars are represented by the standard deviation of the average energies.

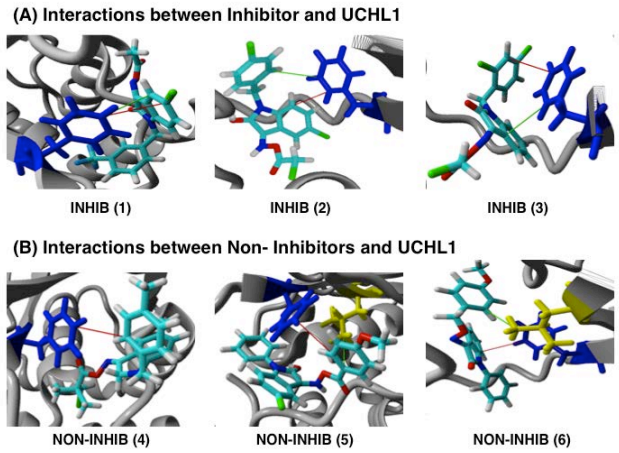

Figure 6. The binding interactions between UCH-L1 and ligands. The hydrophobic interactions are represented by green lines and π-π interactions are represented by a red line. For inhibitors, the hydrophobic interactions and and π-π interactions occur with phenylalanine (Phe) 160 represented in blue. For the last two non-inhibitors, the hydrophobic interactions occur with Leucine (Leu) 52 represented in yellow.

Discussion

From the docking results, the effective binding site where the ligand binds to was found. The binding energy was evaluated for the inhibitors and non-inhibitors that were bound to UCHL1 protein. From the results, the inhibitor that showed activity yielded a higher binding energy that that of the non-inhibitors that showed no activity. In determining cause of the binding energy, it was found that the two effective interactions that contribute to the binding energy are hydrophobic interactions and π-π interactions.

Despite the abundance in UCHL1 protein in the mammalian brain, the molecular mechanism is yet to be resolved. However, this is the first time understanding the molecular pathway of ligand and UCHL1 protein using computational chemistry methods and docking software. These computational analysis and calculations will help understand the interactions between such proteins and ligand, as well as, broaden our understanding on the type of inhibitors that can be best implemented.

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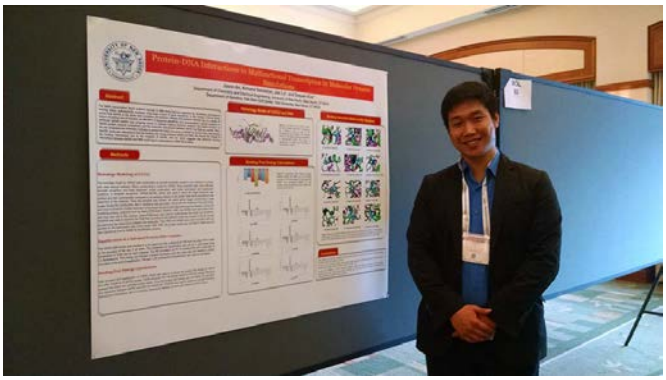
Bibliography

Jason An is a senior majoring in Chemistry and Forensic Science double major at University of New Haven. He is a member of Xiao's research group, where he conducts theoretical and computational chemistry research on the interactions of proteins and ligands under the advisement of Dr. Xiao, a physical chemistry professor in the Chemistry and Chemical Engineering department at University of New Haven. He contributes to the Chemistry department by working as a lab assistant and teaching assistant. Jason returned from Boston, MA where he held a poster session presentation within the biological division of the 250th American Chemistry Society National Meeting and Exposition. His goal is to continue his research in the graduate level and earning a PhD degree in Chemistry. He wants to concentrate his graduate research in areas of Bioinformatics, Organic Chemistry, and Computational Chemistry.



References:

1. Hochstrasser, M., Origin and Function of Ubiquitin-like Protein Conjugation. Nature. **2009**
2. Liu, Y., Lashuel, H., Choi, S., Xing, X., Case, April., Ni, J., Yeh, L., Curry, G., Stein, R., Lansbury, T.P. Discovery of Inhibitors that Elucidate the Role of UCH-L1 Activity in the HI1299 Lung Cancer Cell Line. Chemistry & Biology. **2003**, 10, 837-846.
3. Osaka, H., Wang, L. Y., Takada, K., Takizawa, S., Setsuie, R., Li, H., Sato, S., Nishikawa, K., Sun, J.Y., Sakurai, M., Harada, T., Hara, Y., Kimura, I., Chiba, S., Namikawa, K., Kiyama, H., Noda, M., Aoki, S., Wada, K. Ubiquitin carboxy –terminal hydrolase L1 binds to and stabilizes monoubiquitin in neuron. Human Molecular Genetics. **2003**, 12, 1945-1958.
4. Boudreaux, A.D., Maiti, K.T., Davies, W. C., Das, C. Ubiquitin vinyl methyl ester binding orients the misaligned active site of the ubiquitin hydrolase UCHL1 into productive conformation. PNAS, **2010**, 107, 9117-9122.
5. Grosdidier, A., Michielin, O., Zoete, V. Fast docking using CHARMM force field with EADock DSS. J Comput Chem. **2011**, 32, 2149-2159.