Identifying the Binding Residues on CYP3A4 to Naringin using Protein Modeling and Docking

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Research Question
At which residues on CYP3A4 does naringin bind and how do these residues compare with those at which other inhibitors bind?

Introduction
Cytochrome (CYP) enzymes are a superfamily of monooxygenase hemeprotein enzymes that are found throughout the body but are heavily concentrated in the endoplasmic reticulum and mitochondria of liver cells. These enzymes catalyze reactions that modify a wide range of substrates into more hydrophilic and, therefore, more readily excreted forms. Cytochrome enzymes are heavily involved in the detoxification process of many medically relevant drugs. As such, the importance and activation of these enzymes can substantially alter the effective bioavailability of medications and can introduce additional variables into a pharmacological based treatment. For instance, ritonavir is often administered to improve the bioavailability of certain medications. Cytochrome p450 3AA4 (CYP3AA4) is one of the most abundant cytochrome enzymes and can target a wide range of substrates, including many medically relevant drugs. Inhibition of CYP3AA4 can increase the bioavailability and duration of the availability of medications in the bloodstream. This makes the factors associated with the inhibition and activation of CYP3AA4 of great medical interest and importance. CYP3AA4 has been noted to be inhibited by naringin, a flavanone found in grapefruits and other citrus fruits. However, the characteristics of naringin binding to CYP3AA4 are unknown. Previous studies conducted have introduced insights into the characteristics of the binding of other inhibitors to CYP3AA4. These works have highlighted specific structural and chemical properties that are involved in the binding of inhibitors and substrates to CYP3AA4. The structural binding characteristics of naringin to CYP3A4 were investigated using 3-Dimensional in-silico protein modeling and molecular docking simulations. The results were compared to the characteristics of the binding of other inhibitors to CYP3AA4 to develop and enhanced understanding of the potential mechanisms of inhibition of CYP3AA4.

Materials & Methods

From the RCSB Protein Data Bank database, the PDB file of the human cytochrome p450 3AA4 enzyme was obtained. Despite a bound substrate, the PDB file 8DYC was used due to its recency and extremely high degree of alignment with the apoprotein form. Important features of the protein, such as the active site area and the residues that form the opening of the protein cleft, were identified from various published works detailing the binding of various inhibitors and substrates to CYP3AA4. The specific binding between naringin and CYP3AA4 was identified using molecular docking simulations conducted using PyRx. However, due to computational limitations, the docking did not predict interactions directly with the HEME group. Rather, the final docked structure of naringin to the CYP3AA4 enzyme was produced using the coordinates of the docked naringin and the HEME-containing structure of CYP3AA4. The specific residues at which naringin interacts with CYP3AA4 were identified using Contacts/Clashes in Chimera. The model displaying the interaction between the docked position of naringin and CYP3AA4 was prepared for additive manufacturing using J-mol. The residues identified as involved in the binding of CYP3AA4 and naringin were compared to those identified in the literature.

Discussion

The structure obtained after docking naringin into CYP3A4 reveals structural characteristics and binding residues that provide insight into the potential inhibition mechanism of naringin. The residues Arg 372, Arg 374, Arg 106, Arg 105, Ala 370, Phe 215, Arg 212, Phe 304, Leu 482, Ser 119, Ile 223, Thr 224 were identified to be involved in the binding between CYP3A4 and naringin. The residues Arg 372 and Thr 224 form the opening in the protein that ultimately leads to the active site cleft. Naringin exhibits binding for these residues, suggesting inhibition through obstruction. The remaining residues which naringin leads to CYP3AA4 provides additional support for an obstruction-based inhibition mechanism as many of these residues are thought to form the active site cleft of the protein. Many of the residues found using docking simulations were also noted in previous works examining the interaction between other inhibitors and substrates and CYP3A4. The residues Arg 372, Thr 224, Glu 374, Arg 106, Arg 105, Ala 370, Phe 215, Arg 212, Phe 304 and Ser 119 were noted in the literature, and residues Ile 223 and Leu 482 were not noted in the literature examined and may represent additional residues that may be involved in the inhibition of CYP3A4. The chemical basis by which naringin inhibits CYP3AA4 was not investigated, and the binding interactions between CYP3A4 and the prosthetic HEME group could not be accurately examined. As many other inhibitors act through interactions with HEME, this represents an area for further study to develop a complete understanding of the mechanism by which naringin binds CYP3A4. Overall, the results of the molecular docking suggest that naringin inhibits CYP3A4 by binding and blocking to both the opening of the cleft within which the substrate binds and binding to residues in the active site of CYP3AA4.

Applications

Applications of 3D protein modeling include visual representations of the interactions of CYP3A4 and various ligands. Bioinformatic and biochemical methods were additionally used, creating an enriching learning experience. Specifically, this project aims to depict and compare the location of the binding of naringin in CYP3A4.

Literature Cited


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