Saponins as a Potential PFN2 Inhibitor: In silico Approach

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Research Article

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Saponins as a Potential PFN2 Inhibitor: In silico

Approach

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Abstract

Colorectal cancer (CRC) disease has a high mortality rate and has recently involved human profilin II (Pfn2), an actin-binding protein, as a promoter of its invasiveness and progression. This work evaluated the binding affinity of oleanolic acid saponin over Pfn2 and its structural stability. QM and MM techniques were applied to perform geometrical optimization and calculation of the reactive sites from oleanolic acid, whereas molecular docking and MD simulations for protein-ligand interaction under physiological conditions. Oleanolic acid saponin showed a high binding affinity to the Pfn2 PLP-binding site. Analysis of the protein-ligand structure suggests saponin as a molecule with high potential for developing new drugs against Pfn2 in colorectal cancer cells.

Keywords

Colorectal cancer, profilin, oleanolic acid saponin, quantum mechanics, Fukui, docking, molecular dynamics.

Introduction

Saponins are natural chemicals with many biological activities, distributed widely in plants worldwide¹. Its biological properties have been used in different industries, mainly pharmaceutical, food, farming, and cosmetics²⁻⁵. Saponins' pharmacological interest focuses on its therapeutic activities, including antiarthritic, antirheumatic, anti-inflammatory, expectorant, hypocholesterolemic, diuretic, sedative, antimicrobial, and antitumor properties, and serves as a transporter molecule⁶⁻¹⁰. For example, in several clinical assays, it has been proved that saponins serve as an excellent adjuvant for antigens in vaccines¹¹⁻¹³. Saponins are present as triterpenoid or steroid glycosides, being their structure a determinant factor on its activity. The position of sugar substituents on the molecule and its kind and number of remains can be fundamental in this activity⁹.

The emphasis on profilin research is closely related to its contribution to neoplasia and has increased over the last few years¹⁴⁻¹⁶. Profilins are proteins distributed in all eukaryotic cells. Profilins are associated with the regulation of actin dynamics¹⁷. Profilin 2 (Pfn2) is one of the four isoforms present in humans. Profilin 1 (Pfn1) and Pfn2 are related to neoplastic diseases, different from each one's role in cancer development¹⁴⁻¹⁶. Pfn2 behavior on neoplasms development is varied. In oral squamous cell carcinoma has been found that Pfn2 inhibits tumor growth and decreases aggression¹⁸. Otherwise, in colorectal cancer cells, Pfn2 improves the migration of tumor cells, performing an essential role in metastasis development¹⁹. The development of computational techniques for research on possible therapeutic alternatives has becomes an essential tool. So that, using molecular mechanics and docking tools, we determined the higher affinity and interaction regions that saponins had in Pfn2 protein under physiological conditions.

Computational Details

We have obtained the structure of human Profilin II from the Protein Data Bank²⁰⁻²¹, with the identification code 1D1J. The PDB file contained ligands used for crystallization and additionally contained absent residues, as well as crystallization water molecules. Using the Chimera UCSF computer program²², we have eliminated the ligands molecules used to facilitate crystallization and the water molecules present in the crystal. Additionally, using the MODELLER server²³, the missing residues were completed by homology modeling, completing it with the sequence obtained from the NCBI²⁴. Oleanolic acid is the primary core of saponins; we obtained its structure from PubChem, with identification code CID: 10494. To get the ground state, we performed the energy minimization in the Gaussian 16 program²⁵. In the Density Functional Theory approach, we used the CAM-B3LYP hybrid functional²⁶, which contains a correction Coulombic Adaptive Method for long-range interactions. In addition, we have used the TZVP basis set²⁷⁻²⁹. We have calculated the Fukui functions³⁰ in the deMon2k computational package³¹⁻³². We have used the geometry in the ground state to produce force field parameters in the functional form of OPLSAA; we have utilized server TPPMKTOP for this purpose³³. Oleanolic acid drug-likeness was evaluated by the scan tool Lipinski's Rule of Five in SwissADME³⁴. In addition, the data on absorption, distribution, metabolism, excretion, and toxicity (ADMET) were compared³⁵. The quality of the protein 3D model was evaluated through the Ramachandran plot using the RAMPAGE server³⁶.

We predicted the binding pockets by submitting the structure to DoGSiteScorer³⁷, an automated pocket detection and analysis tool used for protein druggability assessment³⁸. Additionally, we carried out Molecular docking with Patchdock³⁹ and refinement with Firedock⁴⁰⁻⁴¹. The top conformations were selected based on energy and docking scores for further analysis.

We performed the molecular dynamic simulation in GROMACS computational package⁴²⁻⁴³ using OPLS-AA⁴⁴ force field. Likewise, we applied periodic boundary conditions in all simulations with a cut-off distance of 1.1 nm. As well, we used a V-rescale thermostat⁴⁵ to a constant temperature of 309.65K. Also, we used TIP3P⁴⁶ water model and (Na/Cl) ions to solvate and neutralize the system. We evaluated electrostatic interactions in the monopole approximation with particle mesh Ewald (PME)⁴⁷. The time step for integration of the motion equations using the leap-frog algorithm as 2fs. We constrained all bonds involving hydrogen atoms by a Linear Constraint Solver (LINCS). We conducted for 100ns all molecular dynamics. We used a canonical ensemble (NVT) for pre-equilibration and an isobaric-isothermal ensemble (NPT) for equilibration. The package VMD 1.9.1 was used for visualization⁴⁸.

Results and discussion

We selected Oleanolic acid because it shows a 75% compliance with Lipinski's rule of five, being LogP the term reaching a value over the permissible (>5) as shown in Table 1. Also, we performed a complementary drug screening for pharmacokinetics and toxicity properties, as shown in Table 2, from where oleanolic acid shows good absorption at the intestine and blood-brain barrier. On the other hand, it is not carcinogenic but presents toxicity for fish and bees.

The oleanolic acid molecule is a pentacyclic triterpenoid that exhibits anticancerogenic properties. We evaluated its reactivity through electronic density, as shown in Figure 1. Also, we calculated the Fukui functions by differences in the electronic density, obtaining two regions: in the C12 atom for a nucleophilic attack and O69 atom for an electrophilic attack, when we showed electronic density is not clear.

To provide further clarity, we calculated the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) energies and atomic coefficients owing to the importance these two represent for the local reactivity of the molecule. We showed HOMO and LUMO molecular orbitals in Figure 2, referred to as frontier orbitals, and present great importance in chemical reactivity. The HOMO is at -7.7085eV while the LUMO is at 1.2762eV, and the frontier orbital energy gap is 8.9847eV, this frontier orbital accounts for a charge transfer interaction within the molecule and disclose the chemical reactivity over the molecule. Other molecular properties such as ionization potential (I), electron affinity (A), global hardness (η), chemical potential (μ), and global electrophilicity (ω) we calculated too. These results, including HOMO and LUMO energies and gaps, are shown in Table 3.

We uploaded the Human profilin II to DoGSiteScorer achieving to identify six pockets, being pocket 0 the chosen one as shown in Figure 3, because it involves eight residues (TRP3, ILE21, ASP26, TRP31, ALA105, ARG107 LEU134, and ARG135) from where two of them (TRP3 and TRP31) they linked to a poly-L-proline binding site reported on Pfn2.

We performed the molecular docking in the PatchDock server, and we found that oleanolic acid bind pocket 0. To corroborate this binding between oleanolic acid and Pfn2, we used LigPlot, a tool that makes pictorial representations from the ligand and the residues interacting from the protein, as shown in Figure 4.

We checked out the stability of the protein-ligand interactions from two optimized wild and mutant systems: the first considers a profilin simulation with a solvent while the second refers to a docked profilin-ligand docking. The first system contains the crystal structure of Pfn2 with explicit solvation and taking into account the periodicity of the system. The backbone structure of Pfn2 presents B-sheets and α -helix, besides its -N and

-C terminal chains are located close one to the other, and the Ramachandran plot denotes a high probability that this model might be present in nature; this is all shown in Figure 5. The second simulated system from the protein-ligand docking considers the highest affinity pocket for the ligand. We showed RMSD and RMSF calculations in Figure 6, and the first point out an equilibrium state till it reaches the 25ns approximately, from where both systems take different routes, with the coupled system mainly being away from convergence.

On the other hand, we observed that the RMSF graphics show a similar behavior likely by the terminal chains of the protein. However, a significant fluctuation in the mid-chain residues suggests that saponin is interacting with particular residues. As for the radius of gyration, the second system tends to contract; however, its H-bond remains almost constant so that we can state that the high stability of profilins comes from its tendency not to lose hydrogen bonds (see Figure 7).

Saponins are a compound nowadays studied due to their cytotoxic and anticancerogenic properties by stimulating the apoptotic process and non-apoptotic processes such as metastasis inhibition, the breakup of the cytoskeleton angiogenesis, and cellular migration suppression. These properties and the reactivity analysis performed on our oleanolic acid molecule made us think of saponins as a possible drug against colorectal cancer.

Current treatment against colorectal cancer has closely linked to the stage of development in each host. However, most of the directed therapy drugs exhibit several secondary effects. Various groups of researchers have been studying a variety of proteins; one of those is human profilin II (Pfn2), a multifunctional protein that presents two bindings sites: poly-L-proline sequence and actin-binding, besides has reported effects over the migration, self-renewal, and invasion ability of cancer cells in CRC, that is based on the formation of protrusions known as invadopodia that enhance the invasive phenotype(2).

Oleanolic acid saponin arises as a possible inhibitor of this protein due to its several biological properties such as anti-inflammatory, antiviral, and antitumor proven to induce apoptosis and regulate the tumor environment. (1). Indeed, our chemical reactivity evaluation by the Fukui analysis showed that atoms C68 and O69 have an electrophilic character. In contrast, atom C12 has a nucleophilic character, making them likely to interact with others.

Checking out the MD simulation, we found that some of the residues belonging to the poly-L-proline pocket from Pfn2 are involved in the interaction with oleanolic acid. Further, we observed that the tryptophan 3 and 31 from Pfn2 are engaged in electrostatic interactions with C68 and O69 atoms of the molecule. Both molecules, which in effect might hinder the interaction between Pfn2 and other proteins such as WASP, VASP, Ena, N-WASP, are all related to actin filament elongation and induced movement indeed, might affect the invadopodia formation leading to cancer cells invasiveness arrest.

To summarize, Pfn2 is involved in cancer cell invasiveness by its actin-binding affinity, and saponins present an anticancer gene effect on colorectal cancer cells, having a highlight effect over Pfn2 stability confirmation. These results lead to a deeper study on the pharmacokinetics and toxicology of saponins, specially oleanolic acid (OA), needed to develop this molecule as a new drug against colorectal cancer.

Conclusions

We found that oleanolic acid exhibits a reactive site on atom O69, being the EHOMO a negative value showing its tendency to acquire electrons by interacting with others. Besides, OA has a high rate of intestinal absorption and no human toxicity. The active site identification from Pfn2 exhibited six binding sites, pocket 0 the chosen one by its druggability score and residues involved related to its poly-L-proline active site. RMSD

and RMSF structure stability analysis displayed a destabilization from Pfn2 docked with OA compared to native Pfn2, being residues 50-60 and 135-139 involved. Oleanolic acid-binding to the poly-L-proline active site from Pfn2 and avoiding its interaction with actin filament inducing movement proteins arise as an alternative against cell invasiveness in colorectal cancer cells.

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Declarations

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Availability of data and material: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability: Not applicable

Authors' contributions:

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Berly Cardenas-Pillco, Lizbeth Campos-Olazaval, and Jorge Aguilar Aguilar-Pineda. Pamela Lily Gamero-Begazo wrote the first draft of the manuscript, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Table 1. Lipinski rule of five values for oleanolic acid

OLEANOLIC ACID	
Molecular weight	456.70 g/mol
Num. H-bond acceptors	3
Num. H-bond donors	2
Molar Refractivity	136.65
Log P	5.82

Table 2. ADMET properties for oleanolic acid

ADMET properties	OLEANOLIC ACID	
	Value	Probability
Absorption		
The blood-brain barrier (BBB)	+	0.7761
Human Intestinal Absorption	+	1.0000
Caco-2 Permeability	+	0.8353
P-glycoprotein Substrate	Substrate	0.7801
P-glycoprotein Inhibitor	Non-inhibitor	0.7810
Renal Organic Cation Transporter	Non-inhibitor	0.8312
Toxicity		
Human Ether-a-go-go-Related Gene	Weak inhibitor	0.9582
Inhibition	Non-inhibitor	0.8130
AMES Toxicity	Non AMES toxic	0.8490
Carcinogens	Non-carcinogens	0.9394
Fish Toxicity	High	0.9924
Tetrahymena Pyriformis Toxicity	High	0.9850
Honey Bee Toxicity	High	0.8437
Biodegradation	Not ready biodegradable	0.9620
Acute Oral Toxicity	III	0.8316
Carcinogenicity (Three-class)	Non-required	0.5962

Table 3. HOMO, LUMO, and other molecular properties calculated for oleanolic acid

OLEANOLIC ACID	
Molecular Parameters(eV)	B3LYP/6-311G(d,p)
E_{HOMO}	-7.7085
E_{LUMO}	1.2762
$E_{\text{LUMO}} - E_{\text{HOMO}}$	8.9847
Ionization Potential (I)	5.73
Electron Affinity (A)	1.40
Global Hardness (η)	4.33
Chemical Potential (μ)	-3.5677
Global Electrophilicity (ω)	1.4693

Figure 1. A) Oleanolic acid saponin 2D structure shows Fukui function evaluation, where atoms C12 in green possess a nucleophilic character while atoms C68 and O69 in yellow possess an electrophilic character. B) Map of electronic density surface, where blue and yellow regions represent a low and high electronic density, respectively.

A

B

Figure 2. A) HOMO pictorial representation, showing iso values (orbitals with the same energetic value) where green areas possess negative and red positive charges having an E_{HOMO} at -0.28328eV . B) LUMO pictorial representation having E_{LUMO} charges at 0.04690eV , showing iso values from orbitals where green areas possess negative and red positive charges.

Figure 3. Active site identification showing six binding pockets calculated from Pfn2 and describing molecular and chemical characteristics from Pocket 0 that arises as our target pocket.

Figure 4. Molecular docking of human profilin II and oleanolic acid. A) Molecular structure of Pfn2 showing binding pocket with oleanolic acid. B) Plot image of residues involved in the interaction with oleanolic acid at the binding pocket.

Figure 5. Pfn2 optimized and secondary structure. A) 3D representation of Pfn2 being its secondary structure showed up in green (β -sheet) and blue (α -helix). B) Ramachandran plot with a 99.3% (136/137) of all residues in an allowed region ($>99.8\%$) and Lys53 residue (-169.8, -29.5) were the only outliers.

Figure 6. Human profilin II stability analysis comparing wild and docked Pfn2 in red and black, respectively. A) Root mean square deviation (RMSD) from both Pfn2 through 100ns. B) Root mean square fluctuation (RMSF) determines the magnitude of fluctuations from residues of both Pfn2.

Figure 7. A) Radius of gyration shows wild Pfn2 in red and docked Pfn2 in black through 100ns. B) H-Bond determines the stability of the conformation from residues of Pfn2 being wild type in red and docked Pfn2 in black.