SULFATION OF NARINGENIN BY SULFOTRANSFERASES FROM *Arabidopsis thaliana*: AN *INSILICO* ANALYSIS

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SUMMARY

Sulfotransferase (ST) is a family of enzymes responsible for transferring a sulfate group from a donor molecule, typically 3'-phosphoadenosine-5'-phosphosulfate (PAPS), to an acceptor molecule. This process regulates the activity of natural compounds and xenobiotics in the body. Meanwhile, naringenin is a flavonoid widely found in foods such as fruits and vegetables, with various medical applications due to its antioxidant, antibacterial, and anti-inflammatory properties. This study aims to use molecular docking techniques to investigate the interaction between ST and naringenin. In this study, we will employ molecular docking methods to analyze how sulfotransferase and naringenin interact with each other. Following molecular docking using AutoDock Tools, we made predictions to assess the binding potential between ST from *Arabidopsis thaliana* (PDB ID: 1Q44) and naringenin using Discover Studio 3.5. The designed compound showed favorable binding characteristics with naringenin, with key interacting amino acids including Leu 44, Phe 115, Glu 117, Ile 141, Ser 142, Ile 201, Gly 202, and His 208. The binding energy was determined to be -7.99 kCal/mol^{-1}. This binding energy indicates a strong and stable interaction, which is essential for the effective regulation of enzyme activity by the ligand. Therefore, the findings of this study are significant for understanding naringenin as a ligand of sulfotransferase. The strong binding affinity and specific interactions suggest that naringenin could be a potent binder to ST. These results pave the way for further experimental validation and may inform the design of new drugs targeting sulfotransferase. Additionally, this study lays a solid foundation for future efforts in drug discovery and protein engineering, particularly in developing new therapeutic agents exploiting the beneficial properties of naringenin.

Keyword: Arabidopsis thaliana, in silico, naringenin, sulfotransferase.

INTRODUCTION

Sulfation (also known as sulfurylation), is a fundamental biochemical process that mediates essential post-translational modifications of various molecules, including proteins, carbohydrates, and lipids (Gallo *et al.*, 2018). Sulfotransferases (ST) play a pivotal role in regulating biological processes by catalyzing the transfer of sulfate groups from sulfate donors to acceptor molecules, a process referred to as sulfation. These enzymes are key players in catalyzing the sulfation of numerous hormones, neurotransmitters, drugs, and xenobiotic compounds, thereby modulating the activities of diverse endogenous and exogenous substances. Through this mechanism, STs not only regulate biological functions but also participate in adjusting the structure and function of proteins and lipids in the body. In plants, particularly in *Arabidopsis thaliana* - a widely studied model organism in plant biology - sulfotransferases are essential for regulating physiological processes related to growth, defense, and environmental adaptation. *A. thaliana*, a widely studied model organism in plant biology, harbors a plethora of ST enzymes involved in sulfating secondary metabolites within its metabolic pathways. Recent studies have highlighted that these enzymes exhibit high specificity towards sulfate donor molecules, interacting strongly with individual ligands.

Naringenin (4',5,7-trihydroxyflavone), is a natural flavonoid predominantly found in citrus fruits including grapes, oranges, lemons, and grapefruits, with particularly high concentrations in grapefruit peels (Den, Tsiani, 2019). The molecular structure of naringenin includes three hydroxyl groups attached to the flavone backbone, which contribute to its potent biological properties. This structure allows naringenin to interact with various biological targets, affecting numerous physiological processes. Studies have demonstrated that naringenin serves as a substrate for several sulfotransferase enzymes. Through the process of sulfation, naringenin is converted into naringenin-sulfate derivatives, which exhibit distinct biological properties and varying levels of bioavailability. For instance, sulfation can modify naringenin's solubility, stability, and cellular uptake, thereby affecting its biological activity and therapeutic potential. Understanding the molecular structure and molecular interactions underlying the sulfation process of naringenin in plants is of considerable interest due to its significance in plant metabolism, secondary metabolite biosynthesis, and potential applications in agriculture and pharmacology. Despite its low bioavailability, naringenin exhibits numerous promising biological properties with medical significance, including

anti-inflammatory and antioxidant activities (Arafah *et al*., 2020). Additionally, this compound is associated with various beneficial effects.

In this study, we aim to elucidate the molecular mechanisms governing the molecular interactions between *A. thaliana* ST and naringenin using homology modeling and molecular docking techniques. By employing these methods, we seek to gain a deeper understanding of the three-dimensional structure of *A. thaliana* ST and its functions related to the binding and catalysis of naringenin sulfation. Furthermore, molecular docking simulations will allow us to predict the binding modes and binding energies between ST and naringenin, providing valuable insights into the substrate recognition and catalytic mechanism of ST enzymes in plants. The findings from this research are expected to contribute to a comprehensive understanding of the molecular basis of sulfation reactions mediated by ST intermediates and shed light on the structural characteristics and potential binding modes of ST enzymes in plant metabolism. Moreover, elucidating the interaction between *A. thaliana* ST and naringenin may facilitate the design of novel studies in plant secondary metabolite biosynthesis and enhance the production of bioactive compounds with potential agricultural and pharmaceutical applications in daily life.

MATERIALS AND METHODS

Preparation and Refinement of Protein Structure

The sulfotransferase (ST) enzyme from *A. thaliana* was extracted from the Protein Data Bank (PDB) **(Figure 1)** and meticulously prepared for molecular docking studies. Initial processing involved the removal of all ligands using Discovery Studio 3.5 to obtain a pristine, uncomplexed protein structure suitable for detailed analysis. Subsequently, the structure underwent refinement using AutoDockTools 1.5.7 to optimize its suitability for docking simulations.

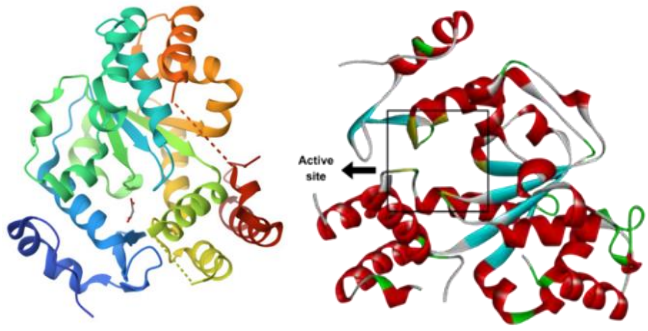

Figure 1. Sulfotransferase enzyme from *A. thaliana*. (A) Crystal structure, (B) Active site region

The refinement protocol included essential steps: computation of Gasteiger charges to accurately depict the protein's electrostatic potential, addition of polar hydrogens to facilitate precise modeling of hydrogen bonding interactions crucial for binding affinity, and merging of nonpolar hydrogens to streamline the structure while maintaining structural integrity and computational efficiency. These methodical refinements ensured the protein structure was well-prepared for subsequent molecular docking studies. The enhanced accuracy in electrostatic potential calculations and representation of hydrogen bonding sites provides a robust foundation for exploring the interactions of sulfotransferase with potential ligands. This rigorous preparation was pivotal for obtaining reliable and reproducible results in docking simulations, contributing to a deeper understanding of the enzyme's binding mechanisms and functional implications. The refined sulfotransferase structure now stands ready for comprehensive investigations into its interactions with specific ligands, such as naringenin, offering insights into potential applications in enzymology and drug design.

Prediction of Active Sites in the Modeled Protein

Accurately predicting active sites in the modeled protein structure is pivotal for comprehending the intricate interactions between Sulfotransferase from *A.thaliana* and its substrate, naringenin. Several sophisticated methodologies are employed for active site prediction, encompassing advanced cavity search algorithms that scan the protein surface, sequence-based analyses aimed at identifying crucial residues, and direct examination of the three-dimensional (3D) structure to locate potential binding sites.

Among these methodologies, PrankWeb emerges as a powerful tool specifically designed for predicting active sites in modeled protein structures. This tool integrates state – of – the – art prediction algorithms with the protein's 3D structure, offering a user-friendly interface that visually highlights potential binding sites. By presenting predictions alongside the protein's sequence and a comprehensive catalog of binding pockets, PrankWeb facilitates immediate and insightful analysis.

The utilization of PrankWeb significantly enhances the accuracy and efficiency of molecular docking studies. It provides researchers with a robust framework to explore and validate potential binding sites, thereby deepening

our understanding of the specific interactions between the sulfotransferase enzyme and naringenin. This comprehensive approach not only aids in unraveling the enzyme's functional properties but also holds promise for guiding rational drug design and enzyme engineering strategies.

Ligand Preparation and Optimization

The preparation and optimization of the ligand, naringenin, initiates with the retrieval of its chemical structure from PubChem, a widely utilized repository for molecular data. This step ensures the accurate representation of naringenin's molecular geometry and chemical properties essential for subsequent computational analyses. Following data acquisition, the ligand undergoes rigorous optimization procedures using AutoDock Tools 1.5.7. The "Prepare Ligand" function within this software suite is employed to minimize energy and adjust bond lengths, thereby enhancing the ligand's conformational stability and geometric accuracy. This optimization step is critical as it ensures that the ligand adopts a suitable conformation for effective binding interactions with the sulfotransferase enzyme. Further refinement and screening of the ligand's structure are facilitated through custom Python 3.8.2 scripts. These scripts provide advanced computational capabilities for optimizing molecular geometry and evaluating physicochemical properties pertinent to ligand-receptor interactions. Such evaluations are essential for predicting and validating potential binding modes and affinity between naringenin and the enzyme. Lastly, the optimized ligand structure is converted into the (.pdbqt) format using AutoDock. This file format is specifically designed for molecular docking simulations, ensuring compatibility with docking software and precise representation of ligand-receptor interactions during computational studies (Huyen *et al., 2023*).

Protein-ligand Interaction Study

The interaction study between the sulfotransferase enzyme from *A. thaliana* and naringenin began by importing the protein molecule into AutoDock 4.2.6. The protein was formatted in PDBQT to ensure compatibility with the docking software. To define docking parameters, PrankWeb was employed to predict active sites, providing crucial information for setting up the GridBox. Defining the GridBox accurately is essential as it guides the ligand towards the protein's binding site during docking simulations. The center coordinates and dimensions of the GridBox were carefully selected to cover the entire three-dimensional structure of the sulfotransferase enzyme. A grid spacing of 0.375 Å was chosen to achieve high resolution in mapping potential binding interactions. The GridBox was centered at coordinates x = 54.534, y = 24.220, and z = 7.374, ensuring comprehensive coverage of the binding site. The dimensions of the grid were set to 70 x 70 x 74 points along the x, y, and z axes, respectively, to explore potential binding interactions thoroughly. The grid parameters were saved in a grid parameter file (GPF).

AutoGrid, a preparatory step in the AutoDock suite, was then used to calculate interaction energies between the protein and probe atoms on the defined 3D grid. Running AutoGrid with the GPF file generated a grid log file (GLG), confirming the successful setup of the grid parameters. Following this, a docking parameter file (DPF) was prepared for the actual docking simulations. This file contained detailed information about the flexible ligand, the grid maps generated by AutoGrid, and docking parameters such as the number of genetic algorithm runs and the maximum number of energy evaluations. AutoDock was executed using the DPF file, converting the docking instructions into a docking log file (DLG). The DLG file provided comprehensive details of the docking simulation, including various binding poses, binding energies, and specific interactions of the ligand at the protein's active site.

The results from AutoDock were meticulously analyzed to identify the most favorable binding interactions between sulfotransferase and naringenin. The docked complexes were ranked based on their binding energies, reflecting the stability and affinity of the ligand-protein complexes. The complex with the lowest binding energy, indicating the most stable and favorable interaction, was selected for further study. This optimal complex was saved in PDBQT format for detailed examination and subsequent analyses.

Docking Results Examination

The docking results were examined using Discovery Studio 3.5 by Biovia, which offers advanced capabilities for analyzing and visualizing protein-ligand interactions in both two-dimensional (2D) and three-dimensional (3D) formats. This comprehensive software provides detailed visualization tools that allow researchers to observe the spatial arrangement and specific interactions between the protein and the ligand.

To gain a thorough understanding of the binding interactions, surface annotation features were utilized to identify and highlight binding pockets and interaction hotspots on the protein surface. These features provided a detailed map of the ligand binding mode, illustrating how naringenin fits within the active site of the sulfotransferase enzyme.

The 2D interaction diagrams generated by Discovery Studio showcased hydrogen bonds, hydrophobic contacts, and other non-covalent interactions between the protein and naringenin. These diagrams are crucial for identifying key residues involved in binding and for understanding the nature of the interactions that stabilize the protein-ligand complex. In the 3D visualizations, the software provided an immersive view of the protein-ligand complex, allowing for a detailed examination of the ligand's orientation and the spatial arrangement of amino acid residues in the binding site. This three-dimensional perspective is essential for appreciating the depth and contour

of the binding pocket and for identifying any conformational changes in the protein upon ligand binding (Chu *et al.*, 2022).

Additionally, Discovery Studio's surface annotation tools highlighted hydrophobic and hydrophilic regions, charge distribution, and the overall topology of the binding site. These annotations helped in pinpointing critical areas of interaction and provided insights into the molecular mechanisms underlying the binding specificity and affinity of naringenin to the sulfotransferase enzyme.

RESULTS AND DISCUSSION

Active site identification

Using PrankWeb, we predicted the active sites within the sulfotransferase protein from *A. thaliana*. This analysis was based on the protein's three-dimensional (3D) structure, focusing on identifying pockets or cavities that are likely to serve as active sites based on their geometric properties, size, and the presence of key residues. The analysis identified several residues contributing to potential active sites, highlighting three main residues: Thr 47, Lys 75, and His 140. Identifying Thr 47 displayed a high probability and significant score, positioning it as a crucial active site component. Lys 75, with its favorable z-score and probability, was identified as another key residue, while His 140 contributed significantly to the structural integrity and interaction dynamics of the binding site. Focusing on these specific residues is essential for comparative binding analysis, enabling researchers to observe how naringenin interacts with the sulfotransferase protein and compare these interactions with those of other ligands. This approach aids in understanding the binding dynamics and structural relationships between the protein and various ligands, which is invaluable for drug design and development. Identifying Thr 47, Lys 75, and His 140 as primary active sites provides a foundation for further investigations into the binding mechanisms of naringenin, offering insights that are crucial for advancing enzyme-ligand interaction studies and informing future therapeutic strategies.

Molecular Docking

A comprehensive molecular docking analysis was conducted to elucidate the interaction between naringenin and the sulfotransferase (ST) enzyme from *A. thaliana*. The primary objective was to understand the binding affinity and interaction dynamics of naringenin with ST, providing valuable insights for future research and potential therapeutic applications. **Table 1** provides detailed information on the interaction and binding energy of the ligand naringenin (4',5,7-Trihydroxyflavonone) with the enzyme sulfotransferase. The docking results revealed that naringenin has a binding energy of -7.99 kcal/mol, indicating a stable and favorable interaction. This binding energy reflects the effective interaction influenced by several types of non-covalent interactions, including hydrogen bonds, van der Waals forces, and Pi-Pi stacking interactions.. Such interactions are crucial as they determine the likelihood of naringenin effectively binding and undergoing catalysis within the enzyme's active site.

Table 1. Interaction and binding energies of ligand with ST

Pubchem ID	Compound Name	Binding Energy Kcal/mol	Interaction	Amino Acid Residues
439246	Naringenin (4′,5,7-Trihydroxyflavonone)	-7,99	2-H Bonds	Thr 47, Lys 75, His 140
			van der Waal	Leu 44; Phe 115, Glu 117, Ile 141, Ser142, Ile 201, Gly 202, His 208
			Pi-Pi and Pi - Alkyl	Ile 50, Pro 74
			Pi-Cation	His 46

The catalytic potential of the sulfotransferase enzyme is highly dependent on the active site residues that facilitate the transfer of the sulfo group. In this study, key residues involved in the catalysis include Thr 47, Lys 75, and His 140. Thr 47 and His 140 are particularly significant as they are directly involved in the catalysis process. Thr 47 plays a critical role in substrate positioning and stabilization, ensuring that naringenin is correctly oriented for efficient catalysis. His 140 is essential for the activation of the sulfo group donor, acting as a proton donor or acceptor during the transfer process, which is a crucial step in the catalytic mechanism. Additionally, Lys 75 is important for maintaining the structural integrity of the active site, which indirectly supports the catalytic activity by ensuring the correct spatial arrangement of the critical residues.

Van der Waals interactions involving residues like Leu 44, Phe 115, and Glu 117 create a supportive hydrophobic environment that further stabilizes the enzyme-substrate complex, further enhancing the efficiency of the catalytic process.These interactions ensure that naringenin remains in a conformation conducive to effective catalysis. Naringenin forms significant hydrogen bonds with Thr 47 and Lys 75, which are essential for maintaining the ligand's position within the active site. Furthermore, Pi-Pi stacking and Pi-Alkyl interactions contribute to the stability and orientation of naringenin, enhancing its binding affinity.

The low docking energy correlates with the presence of strong hydrogen bonds and Pi interactions, which stabilize the enzyme-ligand complex, suggesting that the active site of ST is highly complementary to the molecular structure of naringenin. This complementarity leads to efficient binding and potential catalytic activity. The compatibility between naringenin's size and the ST active site is further evidenced by the favorable docking energy, indicating that the ligand forms optimal interactions without steric hindrance. Visualizations from Discovery Studio 3.5 highlight these interactions in both 2D and 3D diagrams, providing a comprehensive depiction of the spatial arrangement and specific interactions between the protein and ligand. This molecular docking study confirms that naringenin exhibits strong and stable binding to the sulfotransferase enzyme, primarily through hydrogen bonds, van der Waals forces, and Pi interactions, which are crucial for the enzyme's catalytic efficiency.

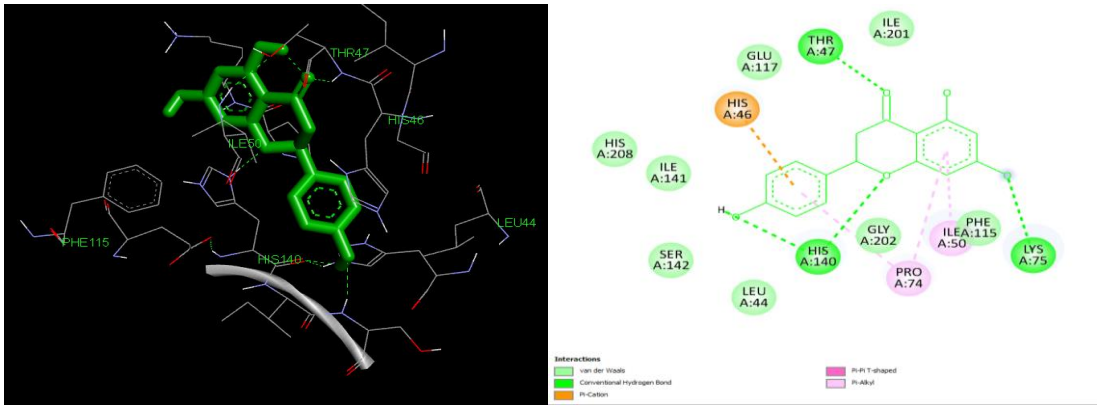

Figure 2. Illustration of naringenin interactions at the active site

(A) 3D: The three-dimensional representation highlights the spatial configuration of naringenin within the active site, showing how it interacts with various residues through hydrogen bonds, van der Waals forces, and Pi interactions, (B) 2D: The two-dimensional diagram provides a clear view of the interaction space, emphasizing the role of key residues in maintaining the ligand's position and facilitating catalysis.

Figure 2 illustrates the interactions between naringenin and the sulfotransferase enzyme at the active site in detail, including three-dimensional (A) and two-dimensional (B) views. The docking analysis results showed that naringenin interacts strongly and stably with the enzyme, with a relatively low binding energy indicating a high interaction between the ligand and the enzyme. The combination of strong hydrogen bonds, hydrophobic interactions, and Pi-cation interactions, particularly at His 46, suggests a robust and stable enzyme-ligand complex. These interactions not only stabilize the complex but also maintain the appropriate conformation of naringenin for effective catalysis. The detailed molecular docking study confirms that naringenin is a strong and stable ligand for the ST enzyme, potentially making it a good substrate for sulfotransferase processes. This discovery could pave the way for new research avenues, particularly in the biological and pharmacological applications of naringenin.

CONCLUSIONS

In this study, molecular docking analysis was employed to investigate the interaction between naringenin and sulfotransferase (ST) originating from *A. thaliana*. The results elucidated the binding capability and interaction characteristics of naringenin with ST, offering valuable insights for further biochemical and pharmacological research. The docking results revealed highly favorable and stable interactions between the ligand and enzyme, assessed through low binding energies and critical interactions, including hydrogen bonds, van der Waals forces, and Pi-cation interactions. Notably, these interactions involved key residues such as Thr 47, Lys 75, and His 140, suggesting that naringenin could serve as an effective ligand for sulfotransferase, potentially influencing its catalytic activity.

These findings underscore the promising potential of naringenin as a biologically active compound in modulating the function of sulfotransferase from *A. thaliana*. The insights gained could be leveraged to design new inhibitors or activators targeting sulfotransferase enzymes, potentially leading to novel therapeutic strategies. Furthermore, these discoveries support the theoretical framework of our model and encourage further research to explore the broader potential functions of ST in drug discovery and protein engineering. Overall, this study highlights the importance of naringenin as a compound of interest, providing a foundation for future exploration in both plant biology and pharmacology, and paving the way for innovative approaches to modulate sulfotransferase activity.

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QUÁ TRÌNH SUNFAT HÓA NARINGENIN BẰNG SULFOTRANSFERASE TỪ *Arabidopsis thaliana*: PHÂN TÍCH *INSILICO*

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TÓM TẮT

Sulfotransferase (ST) là một họ enzyme chịu trách nhiệm chuyển một nhóm sunfat từ phân tử cho điển hình là 3'-phosphoadenosine-5'-phosphosulfate (PAPS), sang phân tử nhận. Trong khi đó, naringenin là một flavonoid được tìm thấy rộng rãi trong các loại thực phẩm như trái cây và rau quả, có nhiều ứng dụng trong y học vì các tính chất chống oxy hóa, chống vi khuẩn và chống viêm của nó. Nghiên cứu này nhằm mục đích sử dụng kỹ thuật lắp ghép phân tử để điều tra sự tương tác giữa ST và naringenin. Trong nghiên cứu này, chúng tôi sẽ sử dụng các phương pháp lắp ghép phân tử để phân tích cách sulfotransferase và naringenin tương tác với nhau. Sau khi ghép nối phân tử bằng AutoDock Tools, chúng tôi đã đưa ra dự đoán để đánh giá khả năng liên kết giữa ST từ *Arabidosis thaliana* (ID PDP: 1Q44) và naringenin bằng Discovery Studio 3.5. Hợp chất được thiết kế cho thấy các đặc điểm kết nối thuận lợi với naringenin, với các axit amin tương tác chính bao gồm acid amin Leu 44; Phe 115, Glu 117, Ile 141, Ser142, Ile 201, Gly 202 và His 208. Năng lượng liên kết được xác định là -7,99 kCal/mol^{-1}. Năng lượng liên kết này cho thấy sự tương tác mạnh mẽ và ổn định, điều này rất cần thiết cho sự điều chỉnh hiệu quả hoạt động của enzyme bằng phối tử. Do đó, những phát hiện của nghiên cứu này có ý nghĩa quan trọng đối với sự hiểu biết về naringenin như một phối tử của sulfotransferase. Ái lực gắn kết mạnh mẽ và các tương tác cụ thể cho thấy naringenin có thể là chất có sự kết nối mạnh mẽ đến với ST. Những kết quả này mở đường cho việc xác nhận thử nghiệm sâu hơn và có thể cung cấp thông tin cho việc thiết kế các loại thuốc mới nhắm vào sulfotransferase. Ngoài ra, nghiên cứu này đặt nền tảng vững chắc cho những nỗ lực trong tương lai trong việc phát hiện thuốc và kỹ thuật protein, đặc biệt là phát triển các tác nhân trị liệu mới khai thác các đặc tính có lợi của naringenin.

Từ khóa: Arabidopsis thaliana, in silico, naringenin, sulfotransferase.

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