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BINDING INTERACTION OF INULOSUCRASE (2YFR) WITH β-D-FRUCTOFURANOSE THROUGH MOLECULAR DOCKING SIMULATION

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ABSTRACT

Molecular docking is used to predict the binding of two or more molecular structures in 3D space and to analyze the distribution of compounds based on their chemical structures and their binding to specific target enzymes or proteins. This study aims to analyze the molecular interaction between the enzyme inulosucrase (PDB ID: 2YFR) and the ligand β-D-fructofuranose using molecular docking. The methods employed were ligand and receptor preparation in Chimera and docking and visualization analysis using AutoDock Tools 1.5.7, AutoDock Vina, and Discovery Studio Visualizer. The docking method was validated using grid box coordinates and the resulting affinity values. The docking results showed that the β-D-fructofuranose ligand has an affinity of –2.9 kcal/mol, indicating a stable interaction and the formation of two strong hydrogen bonds with the active residues ASP42 and GLU45. This indicates the stability of the ligand–receptor complex, suggesting that the β-D-fructofuranose ligand may influence the catalytic activity of the inulosucrase enzyme via a specific hydrogen-bonding mechanism.

ABSTRAK

Molecular docking digunakan untuk memprediksi dua atau lebih sruktur molekul yang dapat berikatan secara 3D untuk menganalisis pola distribusi senyawa berdasarkan struktur kimia dan keterikatan senyawa terhadap enzim atau protein target tertentu. Penelitian ini bertujuan untuk menganalisis interaksi molekuler antara inulosukrase (PDB ID: 2YFR) dengan ligan β-D-fructofuranose melalui pendekatan molecular docking. Metode yang digunakan yaitu preparasi ligan dan reseptor dilakukan menggunakan perangkat lunak Chimera dan proses docking dan visualisasi dianalisis menggunakan perangkat AutoDock Tools 1.5.7, AutoDock Vina, dan Discovery Studio Visualizer. Validasi metode docking dilakukan berdasarkan nilai koordinat grid box dan evaluasi nilai afinitas yang diperoleh. Hasil docking menunjukkan bahwa ligan β-D-fructofuranose memiliki afinitas sebesar –2,9 kkal/mol yang menunjukkan interaksi yang stabil serta dapat membentuk dua ikatan hidrogen kuat dengan residu aktif ASP42 dan GLU45. Hal ini menunjukkan kestabilan interaksi kompleks ligan–reseptor, sehingga ligan β-D-fructofuranose berpotensi mepengaruhi aktivitas katalitik enzim inulosukrase melalui mekanisme ikatan hidrogen spesifik.

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INTRODUCTION

Molecular docking is a method for predicting the interactions and affinities of a compound or ligand with a protein using software. Molecular docking studies are conducted to analyze the interactions between a molecule or compound and the active site of a target protein prior to laboratory experiments (Putri et al., 2024). Enzyme visualization employs an interactive spatial approach to infer relationships between chemical compounds and enzyme targets based on structural similarity and bioactivity data (Donmez et al., 2020). This can help identify the potential of compounds or ligands in their interactions and analyze the implications of protein conformational changes due to interactions with those ligands. One enzyme of interest for studying its activity through molecular docking is inulosucrase, which plays a role in synthesizing inulin and various oligosaccharides used in biotechnology applications.

Inulosucrase is a fructosyl transferase (FTF) enzyme that functions to transfer fructosyl groups from sucrose to acceptor molecules, producing fructooligosaccharides (FOS) or fructan polymers such as inulin (Ghauri et al., 2021). Inulosucrase can catalyze the formation of inulin-type fructans from sucrose substrates by transferring fructosyl groups from sucrose to other acceptor molecules, forming β-(2,1)-fructosidic bonds, resulting in linear fructan chains or inulin (Abaramak et al., 2021). The crystal structure of inulosucrase has three residues involved in substrate binding: Asp272, Asp425, and Glu54, which allow the ligand and receptor to interact, thereby stabilizing the product molecule, such as 1-ketose (Anwar et al., 2012). Mutations in these three residues will affect the ratio between transglycosylation and hydrolysis activities in a reaction to analyze direct interactions with the enzyme's active site, identify the type of bonds formed, and determine the binding affinity of the molecules involved. One ligand with potential for direct interaction with the active site of the enzyme in the catalytic process of fructoside bond formation is the β-D-Fructofuranose ligand.

Although the structural basis of inulosucrase function has been partially revealed, a significant gap remains in understanding its interaction with β-D-Fructofuranose, a relevant intermediate or product analog in the fructan elongation process. Specifically, no detailed molecular docking studies have been reported to characterize the binding affinity, orientation, and stability of β-D-Fructofuranose within the active site of inulosucrase, nor how it may function as an acceptor in further fructosyl transfer reactions. Furthermore, the interaction in the active site residues on the binding behavior of β-D-Fructofuranose has not been explored. This presents an opportunity for further research to better understand the molecular interactions between inulosucrase and β-D-Fructofuranose, which may inform enzyme engineering strategies to optimize fructan synthesis.

METHODS

Materials

The hardware used in this study consisted of a computer system with the following specifications: Acer Aspire ES1-432 laptop operating on Windows 10 Enterprise LTSC 64-bit (version 10.0, build 19044) with 4 GB of RAM. The software utilized included AutoDock Tools version 1.5.7, AutoDock Vina, BIOVIA Discovery Studio 3.1 (Accelrys, Inc., San Diego, CA, USA), UCSF Chimera version 1.9, Command Prompt, and LigPlot+ for comprehensive analysis of the molecular docking process between the ligand and the target protein or enzyme. The materials used consisted of the inulosucrase protein receptor (PDB ID: 2YFR), obtained from the online Protein Data Bank (www.pdb.org), and the ligand β-D-fructofuranose (FRU), which is one of the constituent ligands of the exo-inulinase protein with the PDB ID: 1Y9G.

Methods

Receptor (Protein) Preparation

The 3D structure of the target receptor was obtained from the Protein Data Bank (PDB) through the Research Collaboratory for Structural Bioinformatics (RCSB) website, using the receptor code 2YFR, which corresponds to the crystal structure of inulosucrase. The protein structure was visualized using Chimera or Discovery Studio Visualizer software. Water molecules and native ligands present in the original protein structure were removed, and the modified structure was saved in *.pdb format to serve as the receptor. Subsequently, the 3D protein structure was converted to pdbqt format using AutoDock Tools 1.5.7, in preparation for molecular docking analysis.

Ligand Preparation

The ligand used in this study was β-D-fructofuranose (FRU), derived from the exo-inulinase protein (PDB ID: 1Y9G). The ligand's 3D structure was constructed using Chimera, followed by geometry optimization using the semi-empirical Austin Model 1 (AM1) method implemented in HyperChem Release v8.07. The optimized structure was then saved in PDB format. Subsequently, the structure was modified using AutoDock Tools 1.5.7, where charges were added and rotatable bonds were defined, and finally saved in pdbqt format for molecular docking analysis.

Validation of Docking Methods

The validation of the docking method was carried out using Discovery Studio Visualizer and AutoDock Tools 1.5.7. The process began with the preparation of the receptor and ligand files, which had been previously optimized. The ligand was then docked into the active site of the receptor using a grid box with identical coordinate points and dimensions to ensure consistency in the docking setup. Following docking, the results were evaluated by analyzing the binding energy values and the amino acid interactions formed between the ligand and the receptor. This validation step was essential to confirm that the docking protocol could accurately predict ligand-receptor interactions and was suitable for further docking simulations involving β-D-fructofuranose.

Interaction analysis and visualization

Interaction analysis and visualization were performed based on the results of the bond free energy values generated from the molecular docking that had been conducted. The bond free energy values indicate the strength of the bond between the ligand and the receptor. The lower the bond free energy value, the stronger the bond between the ligand compound and the receptor (Putri et al., 2024). Interaction visualization was performed using the Biovia Discovery Studio program to determine the ligand's position in the enzyme's active site, then further analyzed using the LigPlot+ program to visualize the interactions between the ligand and the analyzed amino acid residues.

RESULT AND DISCUSSION

Molecular docking was performed to determine the interaction of ligands with macromolecules such as target proteins to predict the accuracy and conformation of ligands in the binding site of the target protein or enzyme (Nursamsiar et al., 2020). The docking results showed the affinity and hydrogen bonds formed with the ligands and the active site of the enzyme.

Ligand and Receptor Preparation

Ligand and receptor preparation was performed using Chimera software to prepare the most stable ligand and receptor structures prior to the docking process (Afliana et al., 2024).

(a) **(b)**

Figure 1. Ligand and receptor preparation (a). Inulose receptor (2YFR), (b). β-D-fructofuranose ligand (FRU)

Molecular docking validation

The validation results obtained through the molecular docking method, with interactions between ligands and the active site of the enzyme, are shown by the X, Y, and Z coordinate values of the ligand center in the grid box data generated in Table 1.

Table 1. Grid center coordinates and grid box size

Ligand	*Grid box*	X	Y	Z
β-D-fructofuranose	Center	3.100375	15.412	23.701
	Size	40	40	40

Table 2. Affinity values and interactions between ligands and active site residues of enzymes.

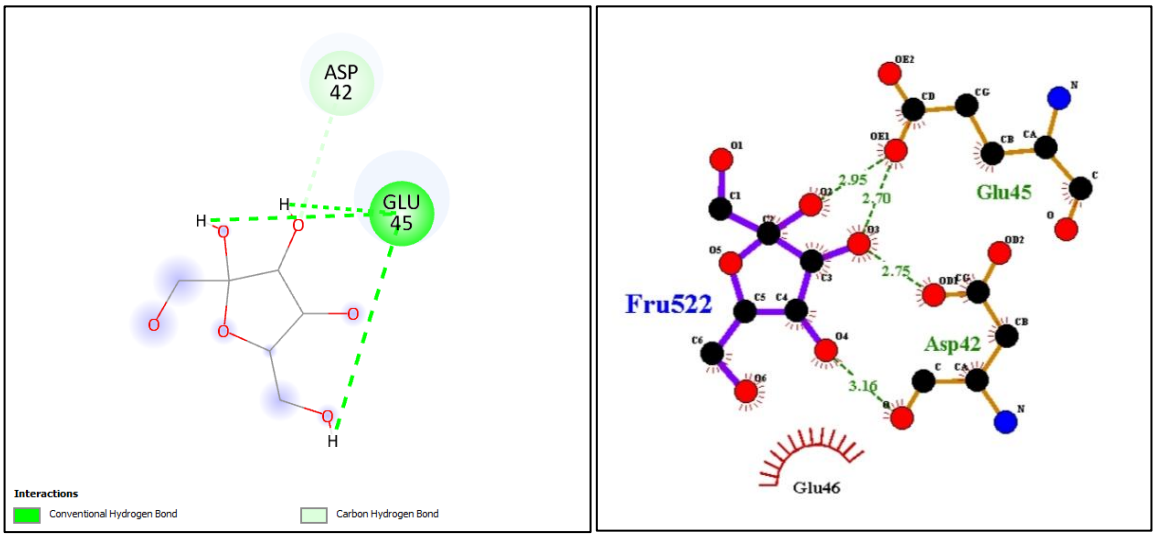
Mode	Affinity (kcal/mol)	Dist from best mode	
		rmsd I.b	rmsd u.b
1	-2.9	0,000	0,000
2	-2.1	1.483	2.834
3	-1.9	1.656	2.331
4	-1.7	2.226	3.058
5	-1.2	3.943	5.917

Table 1 shows the coordinates of the center and the size of the grid box determined based on the location of the active site of the inulosucrase enzyme (2YFR), which is estimated to be the site of the binding reaction between the ligand and the receptor. The results of the docking analysis after determining the grid box coordinates are shown in Table 2, identifying the affinity values and interactions occurring between the ligand and the residues of the enzyme's active site.

Based on the molecular docking results in Table 2, the lowest binding affinity energy value is –2.9 kcal/mol, indicating the most stable interaction between the ligand and receptor. This is because the more negative the binding affinity value, the better the stability of the interaction between the ligand and the target protein (receptor), resulting in a stronger bond formed at the enzyme's active site. The smaller the bond energy or affinity value, the stronger the bond formed between the ligand and the receptor (Putri et al., 2024).

Interaction analysis and visualization

The results of the interaction between amino acid residues and ligands in Table 2 show that the β-D-fructofuranose ligand interacts with two amino acid residues through the formation of hydrogen bonds between the ligand hydroxyl group and the amino acid carboxyl group.



(a) **(b)**

Figure 2. (a) Interaction of ligands with amino acid residues, (b) 2D visualization of ligand-receptor interactions

Table 3. Binding interaction of amino acids and hydrogen bond

Binding interaction (amino acid)	Hydrogen Bond Length (A)
ASP42	1.0099
GLU45	1.01043

The hydrogen bonds listed in Table 3 indicate interactions between the hydroxyl group of β-D-fructofuranose and the carboxyl group of the amino acid residue side chain, forming two hydrogen bonds with the amino acid residues ASP42 and GLU45. These hydrogen bonds play a role in ligand-receptor binding, which can influence the resulting affinity value. The more hydrogen bonds formed, the greater the ligand's affinity for the receptor, thereby increasing the stability and strength of the ligand-receptor complex (Muttaqin et al., 2019). The hydrogen-bond values also indicate strong interactions, given the relatively short hydrogen-bond length of approximately 1.0 Å (10^{-10} meters).

Visualization of the receptor and ligand, based on Figure 2, also shows the interaction of amino acids ASP42 and GLU45 with hydrogen bonds. The amino acid residue Asp42 likely acts as a hydrogen donor from the ligand's –OH group in maintaining the ligand's orientation in the enzyme's active site, while the GLU45 residue functions as a hydrogen acceptor. The interaction between these two amino acids supports the stable placement of the ligand in a configuration that allows the trans-fructosylation reaction involving the β-D-fructofuranose ligand to occur. The interaction between the hydrogen bonds formed from the ligand's hydroxyl group and the amino acid's carboxyl group determines the position of the ligand in the enzyme's active site, thereby stabilizing the enzyme-ligand complex. However, the visualization results also do not show any interaction between one of the main catalytic residues of the inulosucrase enzyme, ASP272, which plays a role in the fructosyl transfer mechanism. This is due to the small size of the ligand, making it impossible to reach the ASP272 amino acid chain as one of the main catalytic residues of the inulosucrase enzyme (ASP272, ASP42, and GLU45).

CONCLUSION

The β-D-fructofuranose ligand exhibits a fairly stable binding affinity to the inulosucrase enzyme (2YFR) with the lowest energy value of –2.9 kcal/mol through molecular docking analysis. The ligand can form two strong hydrogen bonds with the ASP42 and GLU45 residues, stabilizing the ligand-receptor complex. Structural visualization also shows that the ligand can bind to the active site of the main inulosucrase enzyme, although it does not involve one of the main catalytic residues, ASP272. Thus, the results of this molecular docking study provide information on the

potential of the β-D-fructofuranose ligand as a bioactive ligand that can influence the enzymatic activity of inulosucrase in biotechnology applications.

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