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RESEARCH PAPER

Molecular docking study of mutant levansucrase (E342A) from *Bacillus subtilis* as a receptor for D-glucopyranose and β-D-fructofuranose ligands

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Abstract: Molecular docking is a widely applied computational approach for predicting the binding modes of small-molecule ligands within the active site of a target protein. This research investigated the interactions of mutant levansucrase E342A from *Bacillus subtilis* (PDB ID: 1PT2) for β-D-fructofuranose and D-glucopyranose ligands. Using AutoDock Vina, the docking results indicated that β-D-fructofuranose exhibited a higher binding energy of –5.6 kcal/mol, compared to D-glucopyranose with a value of –5.4 kcal/mol, suggesting a more stable interaction. It was supported by the binding interaction analysis of β-D-fructofuranose, which established five hydrogen bonds, including direct interactions with the key catalytic residues Asp86 (2.76 Å) and Asp247 (2.64 Å) that are essential for the enzymatic reaction. In contrast, D-glucopyranose formed four hydrogen bonds, involving Arg360 (3.07 Å) and Glu340 (2.64 Å), with most residues contributing to structural stabilization rather than direct catalysis. These results confirm that β-D-fructofuranose plays a crucial role as a determinant of levansucrase activity in the biosynthesis of levan-type FOS, which are known to exhibit strong prebiotic activity.

Keywords: Levansucrase, molecular docking, β-D-fructofuranose, D-glucopyranose, 1PT2.

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INTRODUCTION

Levansucrase (EC 2.4.1.10) is a fructosyltransferase (FTase) enzyme belonging to the glycoside hydrolase family 68 (GH68). It catalyzes the conversion of sucrose into levan-type fructooligosaccharides (FOS) and levan polymers through β-(2,6)-linked fructosyl residues [1]. FOS are widely recognized as commercial prebiotics and low-carbohydrate dietary supplements due to their resistance to digestion in the human gastrointestinal tract. The biological activity of oligosaccharides depends on their degree of polymerization (DP) [2,3]. FOS, which are short-chain oligomers (DP 2–10), exhibit strong prebiotic activity and are classified as non-digestible oligosaccharides that selectively stimulate beneficial gut microorganisms [4]. One natural source of levansucrase is halophilic bacteria, extremophiles that can survive in high-salinity environments and are known for their considerable biocatalytic potential [5].

Multiple sequence analyses of GH68 proteins have identified several conserved amino acid residues essential for catalysis. Enzymes in this family are widely distributed across different bacterial genera.

Levansucrases from *Bacillus subtilis* (PDB ID: 1OYG) [6], *Bacillus megaterium* (3OM2) [7], *Erwinia amylovora* (4D47) [8], *Erwinia tasmaniensis* (6RV5) [9], and *Gluconacetobacter diazotrophicus* (1W18) [10] have been extensively purified, and their crystal structures characterized through crystallographic and mutagenesis studies. These enzymes share a conserved five-bladed β-propeller catalytic domain that forms a funnel-shaped cavity with a negatively charged centre [11]. The process of sucrose hydrolysis and levan synthesis is driven by a conserved catalytic triad composed of two aspartate residues, which serve as the nucleophile and the transition-state stabilizer, along with a glutamate residue that functions as the general acid/base catalyst [12].

Mutagenesis studies on *Zymomonas mobilis* levansucrase have demonstrated that substitution of Glu278 with Asp or His results in a significant reduction in the catalytic rate (kcat) of sucrose hydrolysis, by approximately 30-fold and 210-fold, respectively. At the same time, substrate affinity (Km) is only slightly affected. These findings confirm that Glu278 is an essential catalytic residue with a dominant role in catalysis rather than in substrate binding [13]. In the case of *B. subtilis*, the E342A levansucrase mutant has been structurally characterized in complex with sucrose. Its crystal structure was determined using high-resolution X-ray diffraction at 2.1 Å (PDB ID: 1PT2). The substitution of Glu342 with alanine (E342A) is of particular interest because it eliminates the negatively charged carboxylate group that plays a critical role in stabilizing interactions with the fructosyl moiety of sucrose [6]. This alteration has the potential to affect both the binding orientation and the stability of the protein–ligand complex. Therefore, molecular interaction analysis of the E342A levansucrase mutant with small molecules is essential to elucidate the specific role of Glu342 in catalytic activity and to evaluate the potential of ligands to form stable complexes with the mutated enzyme.

Molecular docking is a structure-based in silico computational technique widely used to study protein–ligand interactions. This method can predict binding orientation, affinity, and interaction energy between a ligand and its target protein, and has been extensively applied in enzyme mechanism studies and drug design [14]. Additionally, molecular docking is useful for identifying the most stable conformation of a ligand when it interacts with a protein [15]. In this study, D-glucopyranose and β-D-fructofuranose were selected as test ligands for the E342A levansucrase mutant. As natural hydrolysis products of sucrose, these monosaccharides serve as biologically relevant models for evaluating the impact of the mutation on substrate recognition and catalytic efficiency. Previous reports have primarily described the interaction of the E342A mutant with sucrose. Consequently, the present in silico docking analysis was undertaken to evaluate and compare the binding affinities and interaction patterns of D-glucopyranose and β-D-fructofuranose with the catalytic residues. Elucidating these molecular interactions is critical, as they may modulate the catalytic efficiency of the enzyme and shape the biosynthetic mechanism of levan-type FOS, a process in which several key residues are directly involved in polymerization.

MATERIALS AND METHODS

Equipment

Molecular docking was performed on an HP Elitebook 735 G5 with Windows 10 Pro, equipped with an AMD Ryzen 7 PRO 2700U @ 2.20 GHz processor with Radeon Vega Mobile Gfx and 8 GB of RAM. The software used was UCSF Chimera 1.17.3 for protein and ligand preparation, AutoDock Tools 1.5.7 and AutoDock Vina for docking simulations, BIOVIA Discovery Studio 2024 for visualization of results, and LigPlot+ 2.2.4 for analyzing and identifying interacting amino acid residues.

Materials

The materials used in this study were mutant levansucrase (E342A) protein, obtained from the Protein Data Bank website (PDB ID: 1PT2, https://www.rcsb.org/structure/1PT2). The 3D structures of the ligands D-glucopyranose and β-D-fructofuranose were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

Protein Preparation

The research procedure referred to the study by Putri *et al*. (2024) [16] with several modifications. Receptor preparation began by downloading the crystal structure of the mutant levansucrase protein (E342A) with PDB ID: 1PT2 from the Protein Data Bank in .pdb format. The protein structure was processed using UCSF Chimera by removing the native ligand, water molecules, and unnecessary metal ions. Polar hydrogen atoms were then

added, and charges were assigned to the receptor. The prepared receptor was saved as receptor.pdb and subsequently converted into reseptor.pdbqt format using AutoDock Tools 1.5.7 for use in the molecular docking process.

Ligand Preparation

The 3D structures of the ligands D-glucopyranose and β-D-fructofuranose were obtained from the PubChem database in SDF format and then converted into .pdb format. Ligand preparation was carried out using UCSF Chimera, where each ligand was optimized by adding polar hydrogens and assigning charges to obtain the most stable 3D conformation with minimum energy. After optimization, the ligand structures were saved as ligand.pdb and converted into ligand.pdbqt format using AutoDock Tools 1.5.7 to ensure compatibility as input files for molecular docking simulations with AutoDock Vina [16].

Grid Box Setup

The active site coordinates were determined by positioning the test ligand on the receptor using BIOVIA Discovery Studio 2024 to identify the binding pocket accurately. The X, Y, and Z coordinates were then recorded and applied to the grid box created in AutoDockTools 1.5.7. Receptor and ligand files in .pdbqt format were loaded into AutoDockTools 1.5.7, and the receptor was subsequently optimized. The grid menu was selected to define the grid box dimensions by adjusting the X (red), Y (green), and Z (blue) coordinate values, ensuring the ligand was enclosed within the box. The parameters were then saved in the config.txt file and used in AutoDock Vina docking runs [16].

Docking Simulation

Docking simulations were performed using AutoDock Vina, ensuring that the receptor and ligand files were in .pdbqt format and that the parameters in the config.txt file, including the receptor name, ligand, grid centre coordinates (centre X, Y, Z), and grid box dimensions (size X, Y, Z), were properly set. AutoDock Vina, consisting of vina, vina_split, and vina_license, was placed in the same folder to execute the docking process. The docking was run via Command Prompt (CMD), and the results were generated in text format (Notepad). The docked ligand structures were then separated using vina_split.exe. The best model was selected based on the highest binding affinity, indicated by the lowest binding energy (ΔG), which serves as the primary indicator of the interaction strength between the ligand and the target protein [17].

Visualization and Analysis of Interaction

The docking results were analyzed using BIOVIA Discovery Studio 2024 to visualize the ligand position at the enzyme's active site in 3D conformation and the types of interactions formed. Interaction analysis was further complemented with LigPlot+ version 2.2.4, which generated 2D representations to identify amino acid residues involved in hydrogen bond formation and hydrophobic contacts. This visualization step was performed to clarify the interaction patterns and support the interpretation of the docking results [18].

RESULTS AND DISCUSSION

Molecular docking is an in silico approach used to predict how a ligand binds to a protein receptor and to assess the strength of the interactions formed. Through these simulations, it is possible to observe the ligand's position within the active pocket, the types of bonds formed, and the stability of the resulting complex [19]. This study employed the E342A mutant of levansucrase (PDB ID: 1PT2) as the receptor in the docking simulations. This enzyme structure was selected because it has been well characterized through crystallographic analysis, and its catalytic mechanism has been reported. The E342A mutant was chosen as a receptor model since, although it loses catalytic function due to the substitution of Glu342 with alanine, the overall configuration of the active pocket remains preserved, thereby allowing interactions with the test ligands to occur still [6]. Figure 1 shows the crystal structure of the E342A mutant levansucrase in complex with sucrose, determined at a resolution of 2.10 Å.

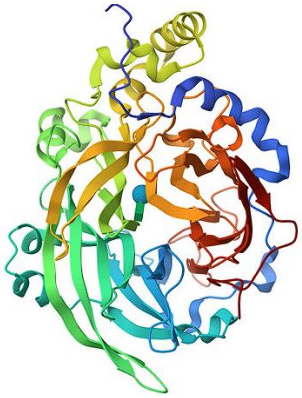
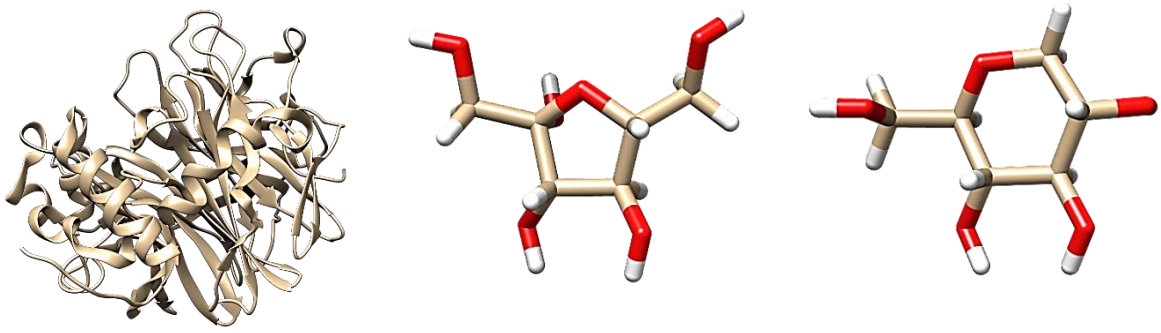


Figure 1. The crystal structure of levansucrase mutant E342A (PDB ID: 1PT2)

The preparation of the receptor and ligands is a crucial initial step in the molecular docking process to ensure accurate prediction of interactions between the ligand and the target protein. The E342A mutation was selected because, although it eliminates the enzyme's natural catalytic activity, its crystal structure retains the integrity of the active site, making it a suitable model for alternative ligand binding. The receptor preparation was carried out by removing non-protein molecules, including water, metal ions, inhibitors, and the native ligand (sucrose), to allow the test ligands to bind at the protein's active site.

Subsequently, polar hydrogen atoms and Gasteiger charges were added using Chimera software. The addition of polar hydrogens was necessary because protein structures derived from crystallography generally lack hydrogen atoms, which are essential for forming hydrogen bonds with ligands. Meanwhile, assigning Gasteiger charges was intended to define the partial charge distribution on protein atoms, thereby enabling more accurate calculation of electrostatic interactions during docking [16]. The optimized receptor structure was then saved in PDB format. The mutant levansucrase E342A receptor is shown in Figure 2A.

Ligand preparation was carried out to ensure that the structures were in optimal condition prior to docking. The ligands used were β-D-fructofuranose and D-glucopyranose obtained from the PubChem database, selected because they are monomers of sucrose, the natural substrate of levansucrase. The selection of these ligands aimed to evaluate the specificity and binding site preference of the enzyme toward each test ligand. Before the simulation, the ligands were optimized by adding polar hydrogen atoms and assigning charges to obtain a minimum-energy conformation, ensuring that the ligands were in the most stable form and that their interactions with the receptor could be modeled more accurately [16]. The results of the ligand preparation for β-D-fructofuranose (Figure 2B) and D-glucopyranose (Figure 2C) are shown in Figure 2.



A B C

Figure 2. Preparation results of (A) receptor 1PT2 (B) ligand β-D-fructofuranose, and (C) ligand D-glucopyranose

Molecular Docking

Determining the coordinates of the active site is an essential step in molecular docking to ensure that the ligand interacts at a biologically relevant location. In this study, Discovery Studio was used to identify the centre of the active site based on the position of the native ligand (sucrose) in the protein crystal structure, while also extracting the centre coordinates (x, y, z) of the binding pocket through an overlay process.

These centre coordinates were then used to construct a grid box with AutoDock Tools. The grid box functions as the search space boundary for the docking algorithm, ensuring that the ligand is evaluated only within regions relevant to the binding site. The determination of grid box size and position is crucial, as it influences the accuracy of interaction predictions [20]. If the grid box is too narrow, the ligand may not be able to accommodate the flexibility of the binding pocket. Conversely, if it is too broad, the docking process becomes less efficient and may generate irrelevant binding poses. Thus, constructing the grid box ensures that the docking simulation is focused on the biologically significant active site, while still providing sufficient space for the ligand to explore its optimal orientation. The centre coordinates and grid box dimensions used for D-glucopyranose and β-D-fructofuranose ligands are presented in Table 1.

In molecular docking studies, the native ligand was re-docked using AutoDock

Vina as a positive control to validate that the docking parameters accurately reproduced the native ligand–protein interactions, thereby serving as a reference for comparison with the test ligands [20]. Binding affinity (ΔG) is a key parameter for evaluating ligand stability, where more negative values indicate stronger and more stable ligand–receptor interactions. Based on thermodynamic principles, a negative Gibbs free energy (ΔG < 0) signifies that the binding process occurs spontaneously. Therefore, ligands with lower binding energy values tend to form more stable complexes with the receptor [21]. The results of the molecular docking simulations are summarized in Table 2, which presents the binding affinity and RMSD values of ligands docked to the levansucrase mutant E342A.

Table 1. Grid centre coordinates and grid box size

Ligand		X	Y	Z
β-D-fructofuranose	Center	40.43	35.415	12.524
	Size	40	40	40
D-Glucopyranose	Center	37.814	37.615	15.921
	Size	40	40	40

The docking results showed that the native ligand (control) had the lowest affinity at -7.6 kcal/mol, which falls within the strong affinity category. It confirms that the natural substrate binds most stably to the active site of levansucrase, consistent with its physiological role. Binding energy serves as the primary parameter for comparing test ligands with the control ligand to evaluate the strength of their interactions. The more negative the Binding affinity, the stronger and more stable the protein–ligand complex formed, allowing the ligand to remain bound to the receptor for a longer duration [16].

For the test ligands, the best binding pose was obtained in mode 1, with β-D-fructofuranose showing –5.6 kcal/mol and D-glucopyranose –5.4 kcal/mol. Both values fall into the moderate interaction category (–5.2 to –5.6 kcal/mol), indicating that β-D-fructofuranose and D-glucopyranose can spontaneously interact with levansucrase mutant E342A, although with lower stability than the native ligand. The small difference in affinity suggests that the two monosaccharides have relatively comparable binding potential, with β-D-fructofuranose exhibiting greater binding flexibility, whereas D-glucopyranose shows more stable binding orientations.

Docking validity was supported by the Root Mean Square Deviation (RMSD) parameter, where values < 2 Å indicate close agreement between the docked and crystallographic conformations, thereby confirming the accuracy of the method used [22]. Overall, β-D-fructofuranose and D-glucopyranose act as potential ligands for levansucrase mutant E342A, forming moderate interactions that may influence enzyme conformation or activity. Although β-D-fructofuranose exhibits a slightly higher affinity than D-glucopyranose, the difference is minimal, and further analysis of amino acid interactions is necessary to determine their binding specificity and functional relevance.

Table 2. Docking results summary

Ligand	Mode	Affinity (kcal/mol)	Dist from best mode	
			rmsd l.b	rmsd u.b
Native ligand	1	-7.6	0.000	0.000
	2	-7.5	2.307	2.421
	3	-7.4	1.784	2.421
	4	-7.3	1.454	4.502
	5	-7.0	2.209	2.655
β-D-fructofuranose	1	-5.6	0,000	0,000
	2	-5.5	5.617	6.847
	3	-5.5	5.580	7.822
	4	-5.5	1.062	3.723
	5	-5.4	2.093	3.867
D-Glucopyranose	1	-5,4	0,000	0.000
	2	-5,3	1.439	2.726
	3	-5,2	1.643	4.487
	4	-5,2	1.561	1.945
	5	-5,2	1.978	3.745

Interaction analysis and visualization

Docking visualization was performed to analyze the interactions between β-D-fructofuranose and D-glucopyranose with the active site of the levansucrase mutant E342A. This method enables detailed examination of the specific interactions formed between amino acid residues and ligands [23]. The observed interactions were mainly non-covalent, including electrostatic forces, Van der Waals interactions, hydrogen bonds, and hydrophobic contacts [24].

The molecular docking results of the levansucrase mutant E342A receptor with β-D-fructofuranose and D-glucopyranose showed that both ligands interact with the enzyme's active site. The docking visualizations presented in Figure 3 show the ligand binding modes as well as the differences in amino acid residues involved in the interactions. The detailed binding residues and hydrogen bond lengths for each ligand are further summarized in Table 3.

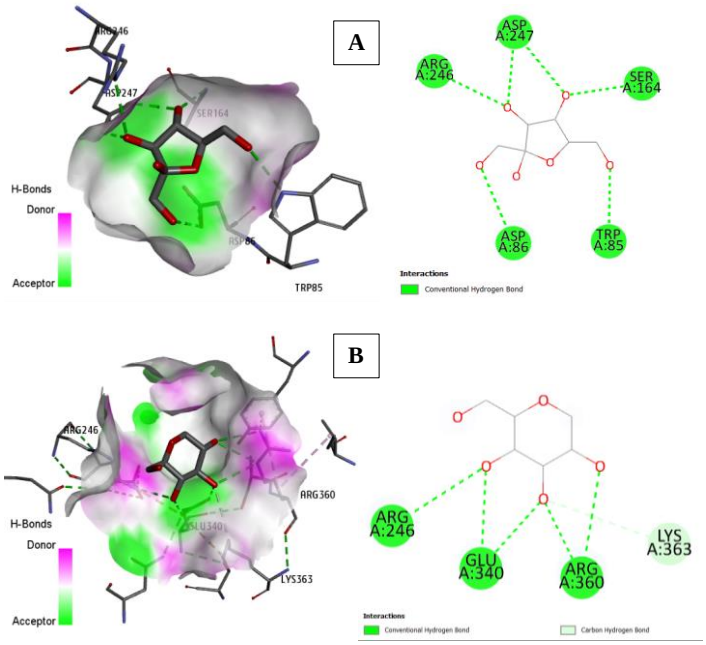


Figure 3. 2D and 3D predicted binding mode from docking simulation of (A) β-D-fructofuranose and (B) D-glucopyranose, into the active site of mutant levansucrase E324A (PDB ID: 1PT2)

Table 3. Results of the interaction between levansucrase mutant E324A and ligands based

Ligand	Affinity (kcal/mol)	Binding Interaction (Amino acid residue)	Hydrogen bond length (Å)
Native ligand	-7.6	Trp85	3.07123
		Ser164	3.19328
		Arg246	3.16127
		Arg360	3.07877
		Glu340	2.64055
		Asp86	2.76799
		Asp247	2.74921
		Lys363	3.67994
β-D-fructofuranose	-5.6	Trp 85	3.07123
		Ser164	3.19328
		Arg246	3.16127
		Asp86	2.76799
		Asp247	2.64833
D-Glucopyranose	-5.4	Arg246	3.31597
		Arg360	3.07877
		Glu340	2.64055
		Lys363	3.67994

The crystal structure of *B. subtilis* levansucrase was first reported by Meng and Fütterer (2003), determined at 2.1 Å resolution for the sucrose-bound complex of the inactive E342A mutant. In this study, sucrose was identified as the native ligand, tightly accommodated within a deep, negatively charged central pocket of the enzyme, which exhibits a distinctive five-bladed β-propeller fold. Structural analyses further revealed the presence of a highly conserved catalytic triad consisting of Asp86, Glu342, and Asp247 that plays a critical role in the enzymatic mechanism.

In accordance with these crystallographic insights, the redocking analysis in this study demonstrated that sucrose, as the native ligand, achieved the lowest binding affinity (–7.6 kcal/mol), consistent with its role as the natural substrate. Docking simulations revealed interactions with several key residues, including Trp85, Ser164, Arg246, Arg360, Glu340, Asp86, Asp247, and Lys363. Notably, the interactions with Asp86 (2.76 Å), Asp247 (2.74 Å), and Glu340 (2.64 Å) fall within the range of strong hydrogen bonds (<3.0 Å), suggesting substantial stabilizing contributions to the sucrose–enzyme complex. These short hydrogen bond distances indicate strong and stable bonds, proving that the docking results are capable of reproducing physiologically relevant interactions. Thus, the presence of bonds in the catalytic triad based on the docking results confirms the validity of the method used and strengthens the biological role of sucrose as a natural substrate for levansucrase.

The docking results presented in Table 3 show that β-D-fructofuranose forms hydrogen bonds with five residues, namely Trp85, Ser164, Arg246, Asp86, and Asp247, with bond lengths ranging from strong to weak interactions (2.6–3.6 Å). The shortest bonds were detected with Asp247 (2.64 Å) and Asp86 (2.76 Å), both of which provide strong stabilizing contributions to the ligand–protein complex. This finding underscores the catalytic importance of these residues and demonstrates that β-D-fructofuranose is able to maintain contact with catalytically essential residues in the active site of the E342A mutant. Hydrogen bonds, which are formed through electrostatic attraction between a hydrogen atom and a highly electronegative atom, represent one of the strongest non-covalent interactions. Shorter bond lengths and a greater number of hydrogen bonds further contribute to the enhanced strength and stability of the complex [24].

The ligand D-glucopyranose formed four hydrogen bonds with the residues Arg246, Arg360, Glu340, and Lys363, with the shortest bond detected at Glu340 (2.64 Å). The absence of direct hydrogen bonds with the main catalytic residues indicates that the binding of D-glucopyranose occurs more peripherally within the active pocket. Instead, its interactions with residues such as Arg360 and Glu340 are more involved in maintaining the structural stability of the catalytic pocket rather than directly contributing to catalysis.

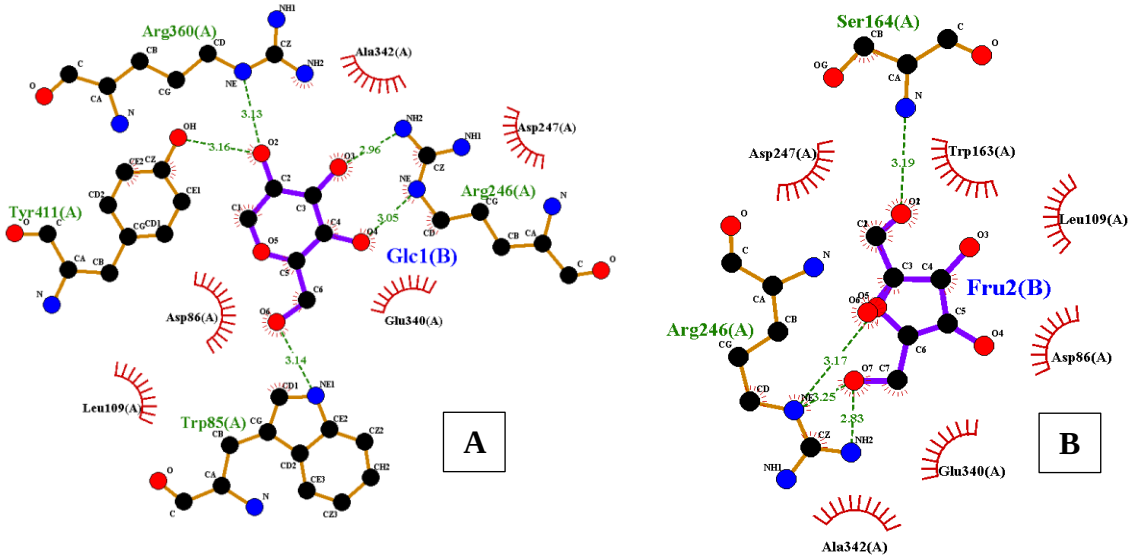


Figure 4. Visualization of levansucrase E342A interactions with (A) D-glucopyranose and (B) β-D-fructofuranose using LigPlot+

The interactions between amino acid residues and the test ligands were further visualized using LigPlot+ to identify key residues involved through hydrogen bonds and hydrophobic contacts [23]. Hydrophobic interactions are non-covalent associations between non-polar groups that minimize contact with the aqueous environment, thereby contributing to the stabilization of protein structures and protein–ligand complexes [25]. These interactions are essential for stabilizing the ligand–protein complex and preserving the conformational integrity of the active site, thus enhancing the reliability of molecular docking predictions.

The structure of the *B. subtilis* levansucrase E342A mutant in complex with sucrose revealed the complete loss of the Glu342 side chain, abolishing its function as the essential proton donor. Despite this substitution, the overall geometry of the active site and ligand orientation remained comparable to the wild-type enzyme. Two water molecules replaced the missing Glu342 carboxylate, while Asp86 and Asp247 retained their nucleophilic and stabilizing roles, respectively. A minor conformational shift, marked by a 17° rotation of Tyr411, was also observed. These findings indicate that although the active-site architecture is largely preserved, the absence of Glu342 eliminates proton donor activity, confirming its indispensable role in the catalytic mechanism of levansucrase [6].

The docking and visualization results demonstrated that β-D-fructofuranose exhibited a slightly higher binding affinity than D-glucopyranose, while the native ligand maintained the strongest binding affinity, reaffirming its role as the primary substrate in the catalytic mechanism of levansucrase. β-D-fructofuranose formed strong hydrogen bonds with the key catalytic residues Asp86 and Asp247, along with additional stabilizing interactions involving Ser164 and Arg246. Its close proximity to the catalytic residues positioned β-D-fructofuranose in a strategic orientation, directly supporting its involvement in the retaining double-displacement mechanism that sustains levansucrase activity. In contrast, D-glucopyranose predominantly interacted with stabilizing residues such as Arg246, Arg360, Glu340, and Lys363, further reinforced by hydrophobic contacts. These interactions indicate that D-glucopyranose functions primarily as a stabilizing ligand or in substrate orientation, rather than as a direct participant in catalysis. Overall, the analysis of binding energies and residue interaction patterns highlights β-D-fructofuranose as the more selective and catalytically relevant ligand compared to D-glucopyranose, although still less effective than the native ligand. These findings provide structural evidence that β-D-fructofuranose plays a greater role in the biosynthesis of levan-type FOS through its direct contribution to fructosyl polymerization.

CONCLUSION

Molecular docking analysis revealed that β-D-fructofuranose and D-glucopyranose can interact with the E342A mutant levansucrase from *B. subtilis* (PDB ID: 1PT2), with binding affinities of –5.6 kcal/mol and –5.4 kcal/mol, respectively. These values indicate relatively stable interactions with no significant difference in affinity. β-D-fructofuranose was further supported by the formation of direct hydrogen bonds with the key catalytic residues Asp247 (2.64 Å) and Asp86 (2.76 Å), which contribute significantly to catalytic activity. In contrast, D-glucopyranose interacted only with structural-supporting residues, such as Arg360 and Glu340, which are more involved in maintaining the stability of the catalytic pocket rather than directly participating in catalysis. These findings suggest that the involvement of key catalytic residues by β-D-fructofuranose has greater potential to influence levansucrase activity in the biosynthesis of levan-type fructooligosaccharides (FOS).

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