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Research Article

In silico approach to investigate the immobilized glucose isomerase from *Lactobacillus reuteri* using biopolymers

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Abstract

Glucose isomerase converts glucose to fructose, and immobilization is a common strategy to improve enzyme properties. Several organic biopolymers (alginate, cellulose, chitin, and chitosan) are potential immobilization supports. Prior to wet-lab experiments, *in silico* analyses were conducted to assess their potential. This study aimed to analyze glucose isomerase from *Lactobacillus reuteri* immobilized on these four biopolymers using computational methods. Homology modeling was performed with SWISS-MODEL, and model quality was evaluated via Ramachandran plot, SAVESv6.1 (ERRAT, Verify3D), MolProbity, and clash score. Immobilization support structures were built using the GLYCAM web server, and docking was performed with PyRx 0.8. Results showed good stereochemical quality: MolProbity 0.82, clash score 0.85, Ramachandran favored regions 97.76% (outliers 0.22%), ERRAT quality factor 99.344, and Verify3D 89.76% (3D-1D score ≥ 0.1). Molecular docking revealed that alginate was positioned farthest from the active site (13.2 Å) compared to cellulose (12.0 Å), chitin (12.7 Å), and chitosan (11.0 Å). Alginate also showed the lowest binding affinity (-7.4 kcal/mol), suggesting minimal interference with substrate binding. In contrast, cellulose (-8.9 kcal/mol), chitin (-9.2 kcal/mol), and chitosan (-9.4 kcal/mol) bound more strongly and closer to the active site, potentially blocking glucose access. In conclusion, alginate is the most promising immobilization support among the tested biopolymers due to its favorable balance of distal positioning and moderate binding affinity, which preserves catalytic activity while enabling enzyme retention.

Keywords: Biopolymer, glucose isomerase, *in silico*, and *Lactobacillus reuteri,* and molecular docking

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1. Introduction

Glucose isomerase is an enzyme that reversibly converts D-glucose into D-fructose. This enzyme is regularly produced by several organisms such as bacteria, fungi, and plants. Glucose isomerase is also called xylose isomerase due to its ability to convert D-xylose into D-xylulose. Chemically, this enzyme requires divalent metal ions to enhance the isomerization reactions. To date, 120 crystal structures have been revealed from various species, especially *Streptomyces* sp. and *Thermus* sp. (Nam, 2022).

Enzyme immobilization has been used as a reliable method to improve enzyme properties. A variety of immobilization support materials have been applied to date, and these support materials must be able to maintain the enzyme's structure and activity (Zdarta et al., 2018). In addition, enzyme immobilization offers several strategic advantages such as high reusability, cost efficiency, and high stability under extreme conditions. Immobilized enzymes were classified into two main clusters, including non-covalent and covalent bonds. Non-covalent bond immobilization involves several interactions, such as van der Waals, hydrogen bonds, or hydrophobic interactions, that link the immobilized support and enzyme surface. Non-covalent immobilization encompasses entrapment, encapsulation, adsorption, and ionic bonding immobilization. However, covalent immobilization needs at least two reactive chemical linkages between immobilized support and proteins. These immobilizations include cross-linked enzyme (CLEs), cross-linked enzyme aggregates (CLEAs), and cross-linked enzyme crystals (CLECs) (Robescu & Bavaro, 2025).

Many biopolymers have been used as supports of immobilization, especially alginate, cellulose, chitin, and chitosan. Those polysaccharides offer several advantages, such as nontoxicity and biocompatibility for many purposes (Anchidin-Norocel et al., 2023). In an immobilized system, alginate will reform the Ca-alginate structure via entrapment formation that increases thermostability and a more extensive pH range (Bilal & Asgher, 2015). While cellulose and its derivatives are included in biomatrices that are highly biodegradable, leading to a low contamination risk to the environment. Cellulose is able to provide covalent or physical immobilization methods (Liu & Chen, 2016), which enhance the efficiency of biocatalysts in various applications, including wastewater treatment and biofuel production. Chitin is one of the most famous biopolymers in nature that is regularly found in the exoskeleton of arthropods. Based on reports, the market for chitin is growing significantly as it offers good stability and cost-effective production (Verma et al., 2020). As a derivative of chitin, chitosan offers several advantages that need to be considered as immobilized supports, such as antimicrobial activity and film-forming ability. In addition, it has functional groups, including amino and hydroxyl, that can enhance the interaction between enzymes and immobilized supports (Ribeiro et al., 2021).

Bioinformatics has been considerably involved in the prediction or discovery of protein-ligand interactions. In general, it can simulate and predict the biocatalysis process in detail; therefore, the application in the lab will reduce time-consuming tasks and increase accuracy (Shuli et al., 2022). Enzyme analysis through bioinformatics to assess the interaction during the biocatalysis process has been applied for decades. However, the notion of enzyme immobilization on biocatalysis was still limited (Padrosa & Paradisi, 2023), particularly in terms of understanding the mechanisms and benefits of using various biopolymers for this purpose. This study aimed to evaluate biopolymers including alginate, cellulose, chitin, and chitosan as potential candidates of immobilized support using *in silico* analyses.

2. Materials and Methods

2.1 Homology modeling of Glucose Isomerase from L. reuteri

The 3D structure of glucose isomerase from *L. reuteri* was not available in the Protein Data Bank (PDB; http://www.pdb.org). The amino acid sequence of glucose isomerase was retrieved from NCBI (ID AAT98631.1). The 3D model of the protein was constructed using the homology modeling server SWISS-MODEL (https://swissmodel.expasy.org/interactive). The stereochemical quality of protein structure was evaluated

via SWISS-MODEL (MolProbity, clash score, and Ramachandran plot analyses) and SAVESv6.1 – Structure Validation Server (https://saves.mbi.ucla.edu/) for ERRAT and Verify3D analyses.

2.2 Constructions of Immobilized Supports

In this study, several biopolymers were used as immobilized supports, including alginate, cellulose, chitin, and chitosan. The structures of these biopolymers as immobilized supports were constructed using the Carbohydrate Builder online server (https://glycam.org/). Each biopolymer consisted of six monomer units, which mimic a complex immobilized support. The original oligomers were converted into PDBQT format using the Open Babel GUI application for molecular docking preparation.

2.3 Molecular Docking

Molecular docking was conducted to estimate the intermolecular interactions between the protein structure of glucose isomerase from *Lactobacillus reuteri* and oligomer ligands. The 3D glucose structure was retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/). Docking simulations were conducted using PyRx 0.8 software (https://pyrx.sourceforge.io/) and visualized with BIOVIA Discovery Studio Visualizer (https://www.3ds.com). The protein structure of homology modeling was docked with glucose as the ligand, and the ligand coordinate was determined using BIOVIA Discovery Studio Visualizer. To investigate the effect of immobilized supports on the protein structure, the protein, glucose isomerase, was docked with each immobilized support construct. The distances between the ligand and immobilized supports were then calculated and assessed using the distance formula below.

The docking grid box was centered on the predicted active site coordinates identified from the glucose binding pose. Specifically, the grid center was defined as the centroid of the glucose molecule docked in the preliminary protein-glucose docking simulation. The grid dimensions were set to 25 Å × 25 Å × 25 Å, which sufficiently encompassed the entire active site pocket while excluding non-specific surface binding regions. The exhaustiveness parameter was set to 8 for all docking runs to balance computational efficiency with sampling thoroughness.

$$\textbf{Distance} = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2 + (z_2 - z_1)^2}$$

3. Results and Discussion

3.1 Homology modeling of Glucose Isomerase from *L. reuteri*

The stereochemical quality of the protein model was evaluated using MolProbity and clash score, resulting in 0.82 and 0.85, respectively. Ramachandran plot analysis indicated that residues were located at the most favored regions, approximately 97.76%, while the outliers were identified as about 0.22% (Figure 1). It showed that the structure has minimum steric conflicts, while the minimum score of ≤ 1 is indicative of a high-quality model. The Ramachandran plot remains a crucial method for assessing protein backbone stereochemistry, with more than 90% of residues in favored regions, indicating high quality (Chen et al., 2010). In addition, ERRAT analysis showed that the model achieved a quality factor of 99.344 (Figure 2), showing a highly reliable protein structure. ERRAT analysis assesses non-bonded atom interactions, with higher scores showing higher model quality (Messaoudi et al.,

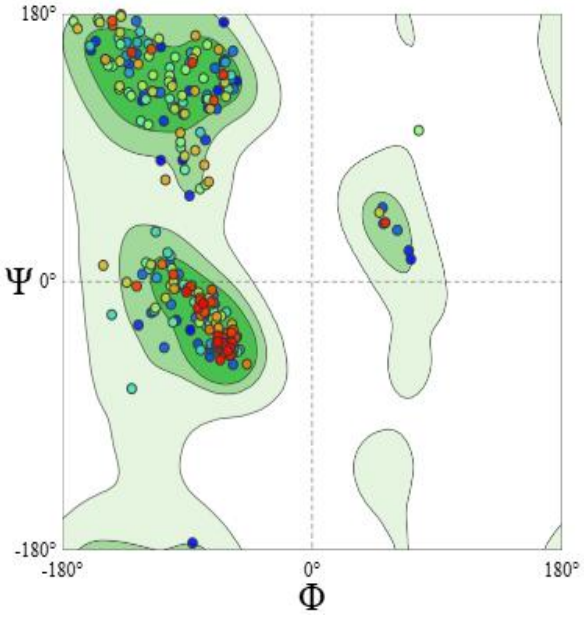

Figure 1. Ramachandran plot of the protein model of glucose isomerase from *Lactobacillus reuteri*

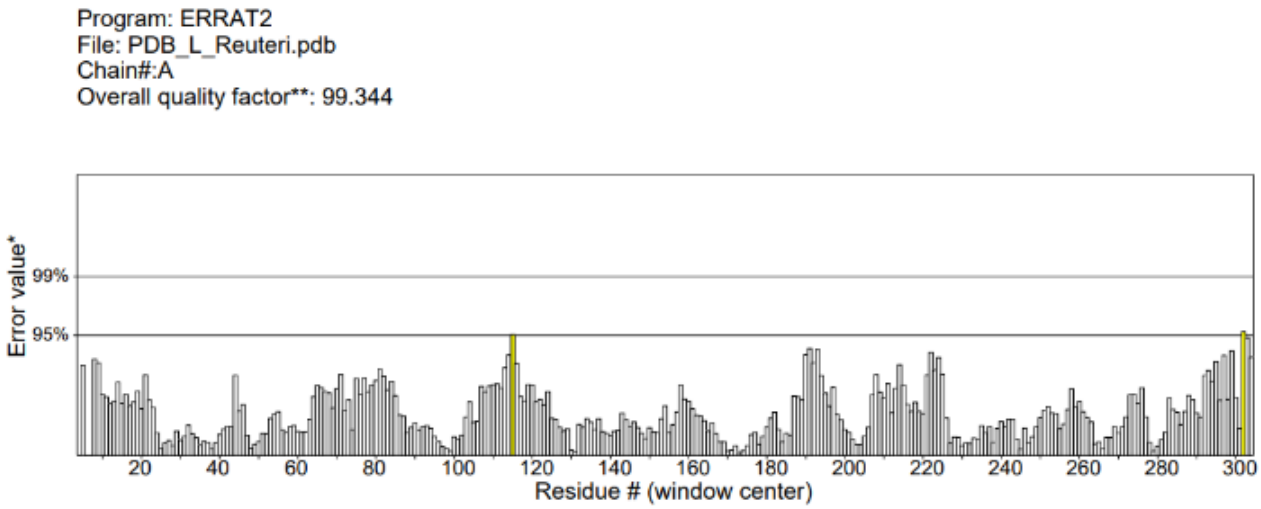

Figure 2. ERRAT2 plot of the protein model for glucose isomerase from *Lactobacillus reuteri*

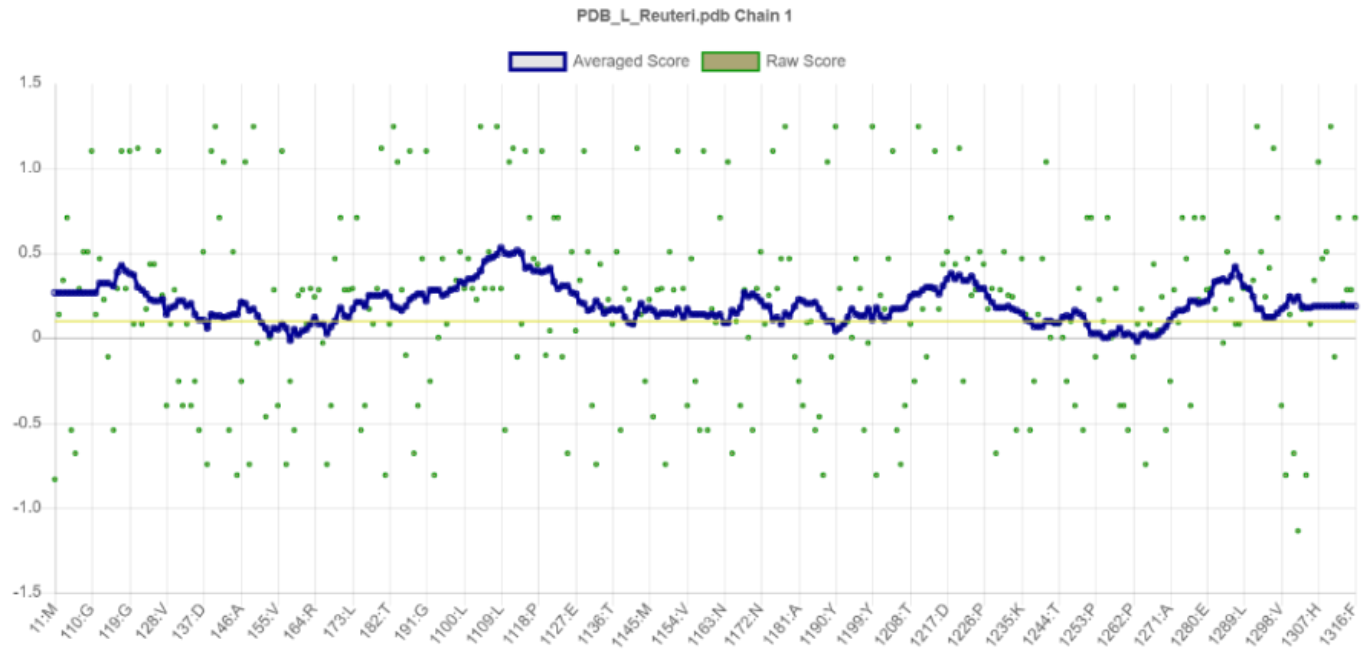

Figure 3. Verify3D plot of the protein model for glucose isomerase from *Lactobacillus reuteri*

2013). Verify3D analysis exhibited that 89.76% of the residues have averaged a 3D-1D score >= 0.1 (Figure 3), indicating an acceptable model. Verify3D evaluates the compatibility between the 3D atomic model and the amino acid sequence (1D), with a score over 80% basically considered reliable (Eisenberg et al., 1997).

3.2 Constructions of Immobilized Supports

Each monomer of the polymers was constructed into oligomers to represent the complex structure of the immobilized support (Figure 4). A previous study reported that six monomers correspond to higher-order multimeric forms, indicating that this number is adequate to represent complex immobilized structures (Khongkomolsakul et al., 2025). Each immobilized support construct was subsequently subjected to simulation by docking with the protein structure.

3.3 Molecular Docking

Molecular docking results showed that among the four immobilized supports, alginate was located farther from the active site, where the ligand glucose bonds, at a distance of 13.232 Å (Table 1). In contrast, the other immobilized supports were located relatively closer to the

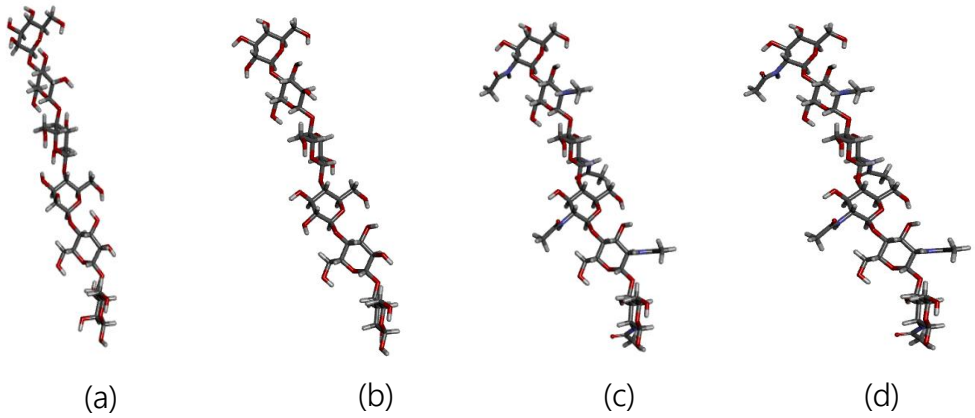

Figure 4. Molecular structures of biopolymers mimicking complex immobilization supports. (a) alginate, (b) cellulose, (c) chitin, and (d) chitosan.

Table 1. Distance between active site of glucose isomerase and immobilization supports.

Protein-Immobilization Agents-Ligands Complex	Coordinate of Immobilization Agents	Coordinate of glucose	Distance (Å)
Glucose Isomerase-Alginate-Glucose	XYZ: 11,381057; 6,147908; -16,804034	XYZ: 5,264941; 3,866706; -5,293941	±13,232
Glucose Isomerase-Cellulose-Glucose	XYZ: 14,534759; 1,945057; -12,795609	XYZ: 5,295882; 3,783118; -5,294176	±12,041
Glucose Isomerase-Chitin-Glucose	XYZ: 15,451705; 2,181410; -12,570124	XYZ: 5,218235; 3,693059; -5,207118	±12,697
Glucose Isomerase-Chitosan-Glucose	XYZ: 13,363257; 2,679295; -12,514962	XYZ: 5,268706; 3,831059; -5,192412	±10,975

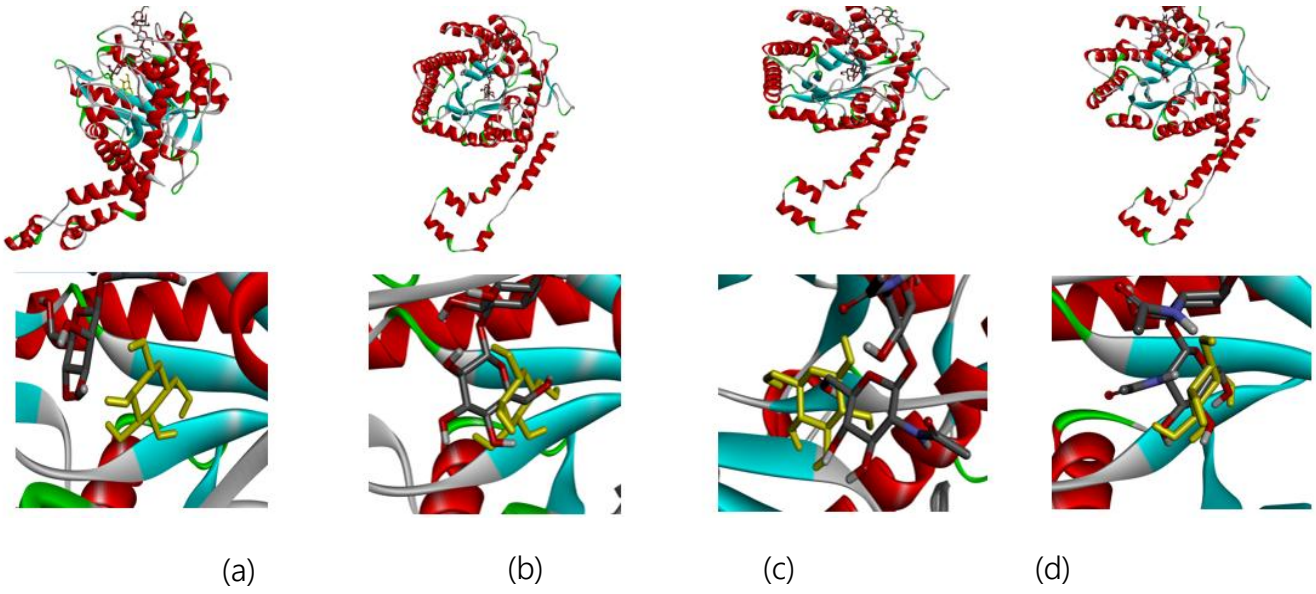

Figure 5. Molecular structures of glucose isomerase – immobilization complexes with glucose. (a) alginate, (b) cellulose, (c) chitin, and (d) chitosan

active site, which may enhance their interference with

protein-ligand bindings. In this study, the glucose (yellowish structure) was visualized as binding precisely to the active site, where the enzymatic reaction occurs (Figure 5). The distant position of the immobilized support prevents it from interfering with the binding of the glucose ligand to the active site of the enzyme. In contrast, immobilized supports located closer to the active site may block the ligand access, thereby inhibiting enzymatic reactions (Garcia‐Galan et al., 2011).

The active site pocket of *L. reuteri* glucose isomerase was identified through structural alignment with homologous glucose isomerase structures (PDB IDs: 1XIB, 1XYL). The catalytic cavity is primarily formed by residues Asp57, Asp59, Lys101, His103, Glu181, Asp245, Lys247, and His269, which are conserved among bacterial glucose isomerases. These residues coordinate the metal ions (typically Mg^{2+}, Mn^{2+}, and Co^{2+}) and facilitate the ring-opening and isomerization reactions. In Figure 5, the glucose ligand (yellow structure) is shown binding precisely within this pocket, with the imidazole ring of His103 (not shown for clarity) positioned to abstract the C2 proton during isomerization.

The analyses showed that alginate indicated the lowest binding affinity among the immobilization supports towards the proteins, with a score of -7.4 kcal/mol (Table 2). This suggests that the probability of alginate blocking the active site of the proteins is lower than that of cellulose (-8.9 kcal/mol), chitin (-9.2 kcal/mol), and chitosan (-9.4 kcal/mol) (Morris et al., 2009). While a binding affinity of -7.4 kcal/mol is considered moderate, it represents an optimal balance for enzyme immobilization: sufficiently strong to retain the enzyme on the support material under operational conditions, yet not so strong as to induce conformational changes or block the active site. In contrast, the stronger binding affinities observed for chitosan (-9.4 kcal/mol) and chitin (-9.2 kcal/mol) may lead to excessive enzyme-support interactions that could restrict substrate access or alter the catalytically active conformation (Sun et al., 2022). Industrial applications of immobilized glucose isomerase, such as high-fructose corn syrup production, typically require enzyme retention under continuous flow conditions at elevated temperatures (60-70°C). The moderate affinity of alginate, combined with its favorable positioning away from the active site, suggests that alginate-immobilized glucose isomerase would maintain catalytic efficiency while achieving adequate operational stability through entrapment rather than strong direct binding.

Table 1. Distance between active site of glucose

Iimmobilized Supports	Binding Affinity (kcal/mol)
Glucose isomerase - Alginate	-7.4
Glucose isomerase - Cellulose	-8.9
Glucose isomerase - Chitin	-9.2
Glucose isomerase - Chitosan	-9.4

4. Conclusion

Among several prominent immobilization supports, alginate is considered a promising support compared to cellulose, chitin, and chitosan. Based on its stereochemical structure, alginate apparently forms structural bonds with the protein at a position farther from the active site, while the other supports are closer to it. Alginate possesses appropriate binding affinity forces to the proteins, whereas the other supports tend to bind more strongly, which may affect catalytic activity at the active site.

Author Contributions

Hary Isnanto: Writing original draft, conceptualization and analysis

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Data Availability

No data was used for the research described in the article.

Declaration of Competing Interests

This study has no conflict of interest to declare.

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