Virtual Screening of Chitosan Derivatives as Potential Inhibitors against *Cassava Linamarase*

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ABSTRACT

Linamarase is a key enzyme involved in the hydrolysis of linamarin, a cyanogenic glycoside present in cassava. Its inhibition is crucial for reducing the release of hydrogen cyanide, a toxic chemical of concern in cassava. In this study, the inhibitory potential of chitosan derivatives against linamarase was evaluated. The binding interaction of linamarase-linamarin complex was first established, followed by virtual screening of chitosan derivatives, which were ranked based on their binding energies and interactions with active site residues. Chitosan derivatives, including N-(carboxymethylidene) chitosan,N-(2-chloro-6-fluorobenzyl) chitosan, N-maleoyl-chitosan, octyl-chitosan, sulfoethyl chitosan, and sulfobutyl chitosan, among others, exhibited the high affinities for linamarase compared with that of the typical linamarase-linamarin interaction. Sulfobutyl chitosan particularly demonstrated the strongest inhibitory potential due to its ability to form stable interactions with key amino acid residues present in linamarase's active site (such as Glu464, Glu406, and Gln193). These findings identify promising candidates for linamarase inactivation from the top performers, offering a potential strategy for cyanide detoxification in cassava, which could significantly enhance the crop's acceptance, shelf life, marketability, and economic value across Nigeria and Africa, thereby promoting food security (SDG 2).

KEYWORDS: Molecular docking, Chitosan derivatives, Cyanide Detoxification, Enzyme inactivation, Food Security.

1. INTRODUCTION

β-glucosidase (EC 3.2.1.21) is a class of glycoside hydrolases (GH) that catalyse the hydrolysis of various glycosides and oligosaccharides.[1] The similarity of amino sequence of β-glucosidases allow for their grouping into families. Cassava (Manihot esculenta) and maize (Zea mays) belong to the GH 1 family with similar amino acid sequence and reactivity.[2] Therefore, β-glucosidase of cassava, linamarase, is structurally similar to that of maize, DIMBOAGlc-hydrolase.

Linamarase, naturally occurring in the cell wall of cassava, catalyses cyanogenic glycosides linamarin and lotaustralin into glucose and acetone cyanohydrin, the latter, which is further broken down into cyanide and acetone.[3] During cassava harvest, contact occurs between the cassava tissues and external elements, which inevitably leads to the wounding of its cell wall, subsequently activating the catalysis of linamarin and lotaustralin by linamarase, thus releasing hydrogen cyanide, a toxic chemical to cassava.[4]

In this study, the inhibitory potential of chitosan derivatives is evaluated against the linamarase enzyme. This is in order to identify the most suitable candidate for linamarase inactivation for limiting or inhibiting cyanide production in cassava.

2. METHODOLOGY

2.1 Ligands and protein preparation

Previously reported chitosan derivatives (85 3D (SDF files) were downloaded from PubChem database (pubchem.ncbi.nlm.nih.gov). The 3D structures were energy-minimized using the MMF94 force field, and then converted to Protein Data Bank Partial Charge and Atom Type (PDBQT) format by Open Babel software.[5] Linamarin was also prepared and saved in pdbqt format, following the same process, the ligand file names were computed in a text file, while linamarase was prepared using AutoDock Tool, sequentially removing water molecules and heteroatoms, while polar hydrogens and Koleman charges were added and then saved in pdbqt format.

2.2 Structure-based virtual screening

The binding site of the linamarase enzyme was assigned on AutoDock Tools by setting the grid box, which corresponds to the active site as reported by,[6] and validated by POcket-CAvity Search Application (POCASA).[7] Thereafter, virtual screening was carried out using AutoDock Vina automated by a Perl Strawberry script. An exhaustiveness of 8 and a number of modes of 10 was set to ensure that different poses of ligands are explored. Consequently, the interaction of each ligand (specifically chitosan derivatives) with the linamarase enzyme were evaluated and ranked based on their binding energies and interactions with active site residues.

3. RESULTS AND DISCUSSION

3.1 Active site determination

POCASA was used to determine the binding pockets of linamarase with a probe radius of 2 Å and a grid size of 1 Å.[7] POCASA unveiled three binding pockets, which were ranked by the volume and volume-depth (VD) value. The volume of a binding pocket depicts the free space available for small molecules to occupy, whereas VD evaluates the potential of a binding pocket to be functional by taking into account its depth. From the three pockets unveiled, 680, 322, and 95, the highest ranked pocket recorded a volume of 1133 Å, and a VD value of 4571 Å (Figure 1a). Comparatively, pocket 680 depicted a volume at least 40 times larger and VD value at least 53 times larger than both pockets 322 and 95, proving pocket 680 as the most likely binding site. Many proteins form pockets when they fold, allowing small molecules to fit in them; these pockets can serve as active sites. The active site of a protein gives more information about its function and structure. Czjzek et al[6]. previously reported the active site of linamarase, showing its opening around loops A, B, C, and D. Glu 406, a nucleophilic part of the catalytic machinery, is located at the bottom of the active site and binds to beta-D-glucopyranose (Figure 1). Czjzek et al[6] also showed that the aglycone-binding pocket contains Phe 198, Phe 205, Phe 466, and Trp 378, which validates the findings from this study.

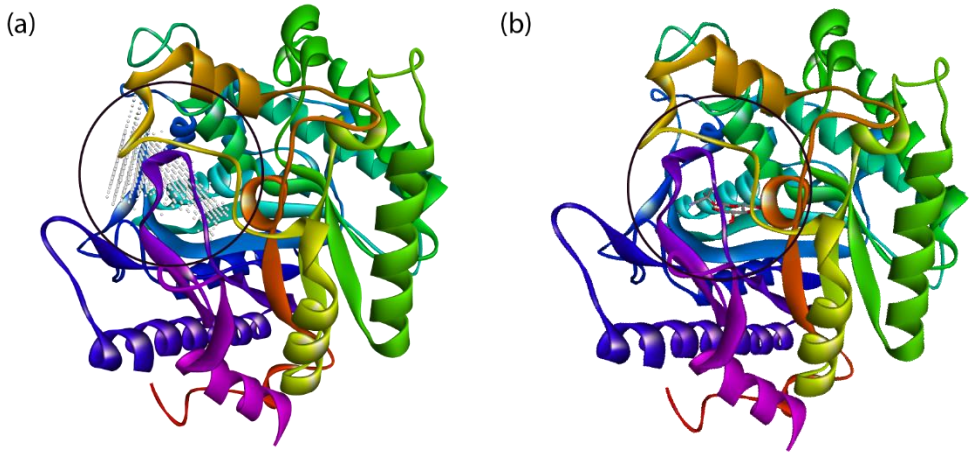

Figure 1: (a) The predicted binding pocket of linamarase, predicted by POCASA (b) Binding site of linamarin with linamarase.

3.2 Structure-based virtual screening

Further to energy minimization of the chitosan derivatives to be analyzed, the eighty-five (85) compounds were screened by docking them at the binding pocket 680 predicted by POCASA.[6] Of the eighty-five chitosan derivatives evaluated, seventy (70) demonstrated a higher binding affinity referenced against linamarin (-7.299 kcal mol^{-1}) (Figure. 2).

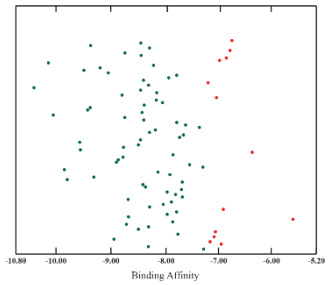

Figure 2: Scatter plot of 85 chitosan derivatives analyzed, showing 70 ligands (≥ -7.746 kcal mol^{-1}) benchmarked against linamarin

Upon further evaluation, chitosan derivatives with close differences from the binding energy of linamarin were screened out, leaving a set of 26 chitosan derivatives that were top performers among all the chitosan derivatives analyzed (Table 1). These chitosan derivatives consistently showed high binding affinities (≥ -7.746 kcal mol^{-1}) across the structure-based virtual screening platform employed. Structure-based virtual screening (SBVS) is a computational strategy used to evaluate how a collection of molecules bind to a target protein by docking them into the 3D structure of the protein.[8] The goal of SBVS is to funnel down a large library of molecules to a smaller list within a short time before performing *in silico* and *in vitro* analysis, which are often much more complex, time-consuming, and expensive. From the virtual screening carried out, a PDB (Protein Data Bank) file was generated, which showed the interaction between each chitosan derivative and the protein, and the type of bond formed between the amino acids

Table 1: Binding affinity of the top 26 chitosan derivatives.

PubChem CID	Chemical name	Binding affinity (kcal mol^{-1})
129721275	N-(2-chloro-6-fluorobenzyl)chitosan	-10.46
129674979	N-(carboxymethylidene)chitosan	-10.19
129830721	N-(3-nitro-4-aminobenzoyl)chitosan	-10.1
132280892	N-(2-thienylmethyl)chitosan	-9.893
135121658	N-(2-azidoethyl)chitosan	-9.84
129853204	N-adamantylethyl-chitosan	-9.608
129865140	N-(2-hydroxybenzyl)chitosan	-9.595
129682035	N-(hydroxypropyl)chitosan	-9.529
129826896	N-(2-(2-pyridyl)ethyl)chitosan	-9.46
129821432	N-(phenylacetyl)chitosan	-9.412
71306969	Carboxymethyl chitosan	-9.406
135121652	N-maleoyl-chitosan	-9.228
129689767	N-succinyl-chitosan	-9.075
129689767	Carboxymethylchitosan	-8.969
169435702	Dimethylethyl amino-chitosan	-8.7
129887062	Carboxy-chitosan	-8.476
129880215	N-sulfo-chitosan	-8.407
129721847	N-methylcarboxy-chitosan	-8.325
129867891	Chitosan oligosaccharide	-8.308
129856284	N-(2-sulfoethyl)chitosan	-8.183
129844296	Chitosan-6-mercaptonicotinic acid	-8.053
129652128	Sulfobutyl-chitosan	-7.979
164186159	Octyl-chitosan	-7.746

Subsequently, further analysis was carried out to understand the amino acid interaction between each of the top performing chitosan derivatives matched against the amino acid interaction between the linamarin-linamarase ensemble. Figure 3 shows the most similar interactions achieved by some of the ligands.

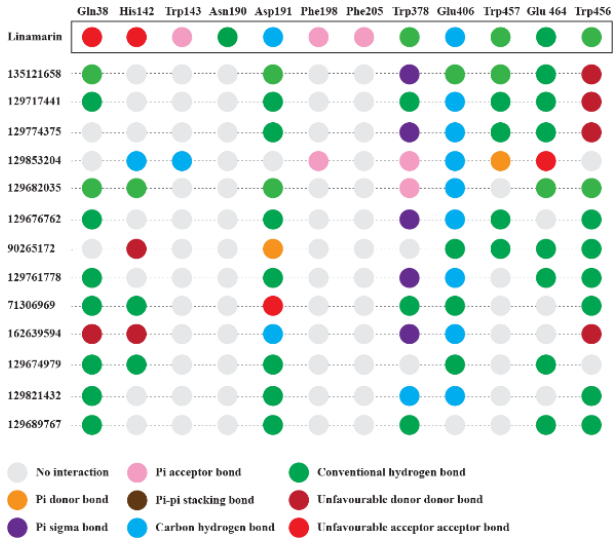

Figure 3: Amino acid interaction of top perming chitosan derivatives

Thirteen (13) of the 26 chitosan derivatives had a C-H bond with at least four of the amino acids linamarin interacts with, validating their strong and potent binding affinities.

4. CONCLUSION

The inhibition of linamarase is crucial for detoxifying cyanide in cassava, a major tropical crop with great potential to address food insecurity. This study successfully evaluated the inhibitory potential of eighty-five chitosan derivatives against cassava linanmarase. Of the eighty-five chitosan derivatives evaluated, seventy (70) demonstrated higher binding affinities than linamarin-linamarase interaction. These were further screened to twenty-six, of which thirteen (13) of these demonstrated strong and similar interactions with salient amino acids involved in the linamarin-linamarase interaction. This validates these chitosan derivatives as potential candidates for inhibiting the cyanogenic activity caused by the hydrolysis of linamarin.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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