THE APPLICABILITY OF THE CRYSTAL STRUCTURE OF *TERMOTOGA MARITIMA* 4-α-GLUCANOTRANSFERASE AS THE TEMPLATE FOR SULOCHRIN AS α-GLUCOSIDASE INHIBITORS

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ABSTRACT

Interaction of sulochrin to active site of glucosidase enzyme of Termotoga maritime has been studied by employing docking method using Molecular Operating Environment (MOE), in comparison with those are reports of established inhibitor α-glucosidase such as acarbose, miglitol and voglibose, and salicinol, as reference compounds. The crystal structure T. maritima α-glucanotransferase (PDB code: 1LWJ) can be employed to serve as the template in the virtual screening of S. cerevisiae α-glucosidase. The comparison between the binding pocket residues of Thermotoga maritima α-glucanotransferase and Saccharomyces cerevisiae α-glucosidase show a high sequence identity and similarity. The result showed that sulochrin could be located in the binding pocket and formed some interactions with the binding residues. The ligands showed proper predicted binding energy (-6.74 – -4.13 kcal/mol) and predicted K_i values (0.011 - 0.939 mM). Sulochrin has a possibility to serve as a lead compound in the development of new α-glucosidase inhibitor.

Keywords: *Docking, sulochrin, α-glucosidase Inhibitor, Thermotoga maritime α-glucotransferase, Saccharomyces cerevisiae α-glucosidase, MOE*

INTRODUCTION

Postprandial hyperglycemia plays an important role in the development of type 2 diabetes and has been proposed as an independent risk factor for cardiovascular diseases [1]. The control of postprandial hyperglycemia is critical in the early therapy for diabetes. One of the therapeutic approaches for decreasing of postprandial hyperglycemia is to retard absorption of glucose by the inhibition of carbohydrate hydrolysing enzymes, for example α-amylase and α-glucosidase [2]. α-Glucosidase inhibitors (α-GIs), have been developed to delay intestinal absorption of carbohydrate. The α-GIs bind competitively to the carbohydrate-binding region of α-glucosidase enzymes, thereby competing with oligosaccharides and preventing their cleavage to absorbable monosaccharides [3].

Screening of glycosidase inhibitors is becoming increasingly prevalent because glycosidases are responsible for the processing and synthesis of complex carbohydrates, which are essential in numerous biological recognition processes. Inhibitors of these enzymes are important molecular tools for glycobiology and can be used to modulate cellular functions [4]. There are reports of established α-glucosidase Inhibitors such as acarbose, miglitol, and voglibose from micro-organism [2,5] could effectively improve hyperglicemia as well as diabetic complication [6], and salacinol a potent antidiabetic from Ayurvedic traditional medicine *Salacia reticulate* [7-8]. Moreover, these inhibitors are recently being investigated in the development of drugs for obesity, HIV infections and HIV-mediated syncytium formation in vitro [5], viral attachment, and cancer formation [9].

Sulochrin (2-(2,6-Dihydroxy-4-methyl-benzoyl)-5-hydroxy-3-methoxy-benzoic acid methyl ester) was obtained from solid state fermentation of *Aspergillus terreus* which reported active as α-glucosidase inhibitor (IC_{50} value 8.5 µg/mL) and possible to used as lead compound for designing new potent inhibitor for glycosidases [10-11]. Based on *in vitro* activity of sulochrin as α-glucosidase inhibitor, within this project, binding site energy (ΔG) was measured by *in siliko* method (docking) between sulochrin as an active ligand and *T. maritime* α-glucotransferase enzyme [12] as receptor. This ΔG value is expected to be based value for designing sulochrin derived compound with improved activities as α-glucosidase inhibitor

The biological tests of α-glucosidase inhibitors were mostly carried on *S. cerevisiae* α-glucosidase [1-2,6]. Unfortunately, the crystal structure of *S. cerevisiae*

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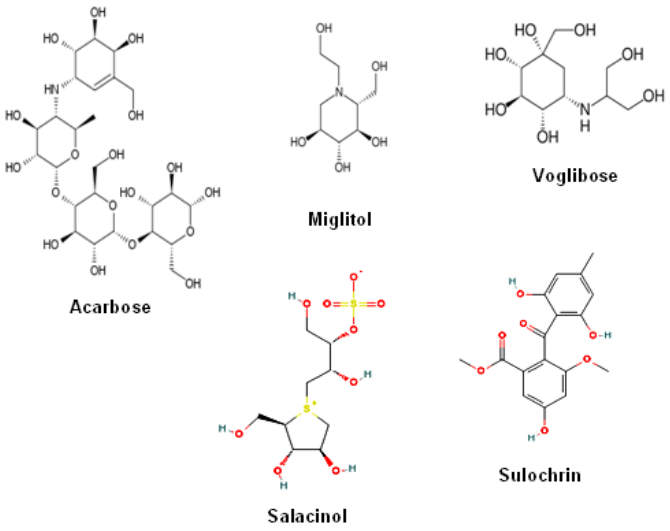

Figure 1. α-glucosidase inhibitors

α-glucosidase has not available yet. Some efforts to build the homology model have been performed and used the crystal structure of *T. maritima* α-glucanotransferase to analyze and compare the ligand-protein interactions. The binding pocket residues of both enzymes were reported show high sequence identity and similarity [13-14].

In this article, we present the amino acid sequences alignment of the binding pockets of both enzymes and the docking analysis of sulochrin compare with established α-glucosidase inhibitors such as acarbose, miglitol, voglibose, and salacinol (Figure 1) by employing Molecular Operating Environment [15]. The sequence alignment and docking studies have been performed to investigate the applicability of the crystal structure of *T. maritima* α-glucanotransferase to serve as template for virtual screening of α-glucosidase inhibitors as well as to study the ligand-protein interaction of the α-glucosidase inhibitors to *T. maritima* α-glucanotransferase and developed sulochrin analog.

EXPERIMENTAL SECTION

Material

Structure of *T. maritima* α-glucanotransferase, by A. Roujeinikova *et al.* to the protein data bank website (http://www.pdb.org/; PDB code: 1LWJ) [12] was obtained and employed as the virtual target.

Instruments

Molecular Operating Environment (MOE) version 2008.10 (developed by Chemical Computing Group Inc, Canada) [15] were employed to perform the docking procedures. All computational simulations were performed on a Linux (Ubuntu 8.04 LTS Hardy Heron) machine with Intel Core 2 Duo (@ 2.5 GHz) as the processors and 3.00 GB of RAM.

Procedure

Sequences alignment of the binding pockets

The *S. cerevisiae* α-glucosidase amino acid sequences (Swiss-Prot code: P53341) was aligned to the *T. maritima* α-glucanotransferase amino acid sequences (PDB code: 1LWJ) using default setting of the align module in the Sequence Editor of Molecular Operating Environment (MOE) version 2008.10 (developed by Chemical Computing Group Inc, Canada). The binding pocket of *T. maritima* α-glucanotransferase defined by MOE as the residues within 4.5 Á from the ligand. The identity and similarity of the binding pockets of both enzymes were then analyzed.

Docking procedures

The crystal structure *T. maritima* α-glucanotransferase was used in the docking analysis (PDB code: 1LWJ) by employing MOE. Hydrogen atoms were added and partial charges were calculated for each atom. The default MM settings were used (MMFF94x). The original ligand in the *T. maritima* α-glucanotransferase was docked in order to check the validity of the docking procedures. In the docking of the original ligand, the default settings were applied, except the followings: The option “Rotate Bonds” was deselected, and the “Affinity dG” algorithm was used as the scoring function. The structures of acarbose, miglitol, and voglibose were obtained from DrugBank with the DrugBank IDs DB00284, DB00491, and DB04878, respectively. The structures of salacinol, and sulochrin were obtained from PubChem with the PubChem IDs 6451151 and 160505, respectively. Hydrogen atoms were added, and if there was a basic amine moiety in the structure, the amine was protonated. The structures were objected for the conformational search and energy minimization. The structures were then docked to *T. maritima* α-glucanotransferase. The default settings were applied, except the scoring function. The “Affinity dG” (ΔG) scoring function was employed.

RESULT AND DISCUSSION

Sequences alignment of the binding pockets

The binding pocket of *T. maritima* α-glucanotransferase consists of 27 residues. The sequence alignment of the residues to the *S. cerevisiae* α-glucosidase amino acid sequences showed circa

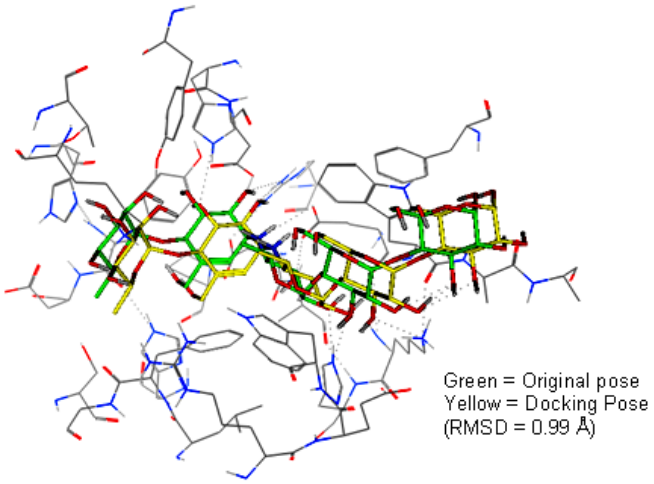

Figure 2. The pose of the original ligand of the *T. maritima* α-glucanotransferase

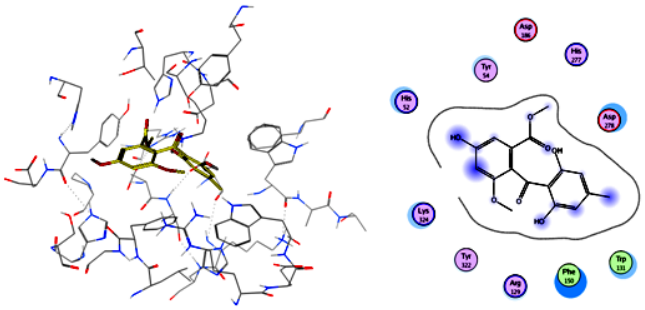

Figure 3. Sulochrin shows high affinity to the enzyme

Tables 1. the *S. cerevisiae* α-glucosidase ligands can be located inside the binding pocket of the *T. maritima* α-glucanotransferase with a proper ΔG

Compound	Predicted ΔG (kcal/mol)	Predicted K_i (mM)
Acarbose	-4.13	0.939
Miglitol	-4.86	0.274
Voglibose	-4.41	0.585
Salacinol	-6.74	0.011
Sulochrin	-6.01	0.039

52% sequence identity and 85% sequence similarity. Remarkably, the binding residues of the original ligand of the *T. maritima* α-glucanotransferase showed 100% sequence identity.

Docking of known ligands

The docking procedure could reproduce the pose of the original ligand of the *T. maritima* α-glucanotransferase with root mean square deviation (RMSD) of 0.99 Å (Figure 2) and predicted binding energy (ΔG) value of -7.05 kcal/mol, which was equal to predicted K_i value of 6.80 μM. The docking studies of known *S. cerevisiae* α-glucosidase ligands showed that the ligands could be located inside the binding pocket of the *T. maritima* α-glucanotransferase with a proper ΔG (Table 1). Notably, this study revealed that salacinol and sulochrin could serve as potential lead compounds for *T. maritima* α-glucanotransferase inhibitors as well as for *S. cerevisiae* α-glucosidase.

The comparison between the binding pocket residues of *T. maritima* α-glucanotransferase and *S. cerevisiae* α-glucosidase show a high sequence identity and similarity. The similar phenomenon has been reported on the human histamine H_3 and H_4 receptors. In fact, most ligands of human histamine H_3 receptor were reported to have high affinity on human histamine H_4 receptor. Since the residues in the binding pockets share high sequence similarity and the docking procedure can reproduce the pose of the original *T. maritima* α-glucanotransferase ligand, the crystal structure *T. maritima* α-glucanotransferase (PDB code: 1LWJ) can be employed to serve as the template in the virtual screening of *S. cerevisiae* α-glucosidase.

In order to confirm the applicability of the crystal structure *T. maritima* α-glucanotransferase (PDB code: 1LWJ) to be employed as the template in the virtual screening, some known *S. cerevisiae* α-glucosidase ligands were tested. The result shows that the ligands can be located in the binding pocket and form some interactions with the binding residues. The ligands show proper predicted binding energy (-6.74 – -4.13 kcal/mol) and predicted K_i values (0.011 – 0.939 mM). Moreover, sulochrin shows high affinity without any hydrogen bond or any electrostatic interaction to the enzyme (Figure 3). This means that designing new ligands by employing sulochrin as a lead compound can be a promising approach. The ligands should adapt the scaffold of sulochrin and have additional substituent that can form hydrogen bond or electrostatic interaction with the protein.

CONCLUSION

The crystal structure *T. maritima* α-glucanotransferase (PDB code: 1LWJ) can be employed to serve as the template in the virtual screening of *S. cerevisiae* α-glucosidase.. The result showed that sulochrin could be located in the binding pocket and formed some interactions with the binding residues. Sulochrin, therefore, has a possibility to serve as a lead compound in the development of new α-glucosidase inhibitor.

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