Analysis Interaction of Glucosyltransferase Inhibitor of Caries from Fatty Acid by Molecular Docking Simulation

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The most common human oral disease is the oral infection dental caries. Dental caries mostly caused by S*treptococcus mutans* that produces extracellular glucosyltransferases (GTFs) that synthesized glucan from sucrose. These glucan is important in determining the permeability properties and adhesiveness of dental plaque. In order to prevent synthesis of glucan formation, inhibitor substances is needed to block the activity of enzyme glucosyltransferase. Nowdays the enzyme inhibitors are used to prevent dental plaque formation are not optimally effective, so the new emerge natural substances is need to develop. In this research, we have conducted *in- silico* study to analysis of Oleic acid, Palmitic acid, linoleic acid and linolenic acid from coconut oil, which has a role as GTFs inhibitor of dental caries. The docking result identified that oleic acid have greater affinity and ability to inhibit of GTFs, this affinity oleic acid complexs is 7.262 µM and energy minimized is -5.6863 Kcal/mol . They have residues contact of OH binding to GTFs are 2 formed hydrogen binding in catalytic site lys228 with score 28.7 % and H distance 2.61 A^o , gly315 with score 35.8 % and H distance 2.77 A^o. The docking result showed that oleic acid has better binding energy and affinity than other bioactive compounds.

Keywords: Glucosyltransferase(GTF), Fatty acid, Caries, Docking simulation

Introduction

Dental caries is the medical term for tooth decay or cavities. It is caused by specific types of bacteria. They produce acid that destroys the tooth's enamel and the layer under it, the dentin. *Streptococcus mutans* has been strongly implicated as a causative organisms of dental caries. The ability of this bacteria to bind to high-molecular-mass glucans synthesized from sucrose is recognized as an important determinant in the formation of dental plaque and tooth decay (Hamada & Slade, 1980)[a]. This organism synthesizes water-insoluble glucan from sucrose with its cell-free and cell-bound glucosyltransferases (GTFs) (Montville, *et al.* 1978). De novo synthesis of glucan is essential for the adherence of *S. Mutans* to tooth surface (Hamada & Slade, 1980)[b]. Many chemical and enzymatic procedures for eliminating *S. Mutans* from tooth surfaces have been explored. However, there are few reports concerning substances that inhibit GTFs activity. Thus, the protein that enable bacteria to bind glucans have attracted interest as potential targets for inhibition of the caries process. Using GTF inhibitors is considered to be a useful means of preventing glucan formation without disturbing the balanced of helpful oral bacteria. In the present study, we developed an agent that can be used either as food or a medicine for safely treating decaying teeth by docking simulation of several fatty acids *In silico*.

In silico is used in the screening process of bioactive compounds or molecular simulation as a drug (Kitchen, *et al.* 2004). Analysis was based on Gibbs energy values, inhibition constants (Gohlke, *et al.* 2000), conformation of the structure, affinity, and hydrogen bonding of enzyme and ligands (Datta, 2002).

Materials and Methods

Materials of this research such as: Protein structures may be downloaded from the site with specific keywords or a PDB alphanumeric filename. The ligands of fatty acid performed using ACD Lab software. MOE software: The docking experiments were performed using the docking software MOE 2008.

Enzyme used in this study is GTFs. GTFs sequences were obtained from complete sequences from *Streptococcus mutans* bacteria. Complete sequences were downloaded from NCBI (http://www.ncbi.nlm.nih.gov/genomes/flu/). Multiple sequence alignment method was based on the ClustalW-program (www.ebi.ac.uk/Tools/clustalw2/index.html website). Homology modeling was performed using the Swiss model which can be accessed through http://swissmodel.expasy.org/SWISS-MODEL.html. A three-dimensional structure was obtained that has the highest similarity with GTFs sequences from PDB (Protein Data Base) with code number 1YRO.

Ligand docking process performed on the fatty acid (linoleic acid, oleic acid, palmitic acid, linolenic acid, oleic methyl, and palmitic methyl) against the receptor. Docking process is performed only on all the amino acids of the receptor. In the process of docking receptors are rigid conditions while the ligand will be conditioned on the state of a flexible so that it can freely move and rotate. The parameters set in the docking process involve setting the scoring function using dG London. Scoring function to measure the biological activity by binding and interactions that occur between the ligand with the target protein (Nylander, 2007).

Molecular docking. For getting the ligands-receptor binding energy procedures of molecular docking were followed. The detailing of the procedure is as follows.
Preparing the ligand and macromolecule files for MOE we prepared the files as follows:
(a) *The Macromolecule file*: The downloaded PDB files were first read in MOE, added waters removed and polar hydrogens were added. And Geometry optimization and minimization receptor Geometry optimization and energy minimization of three-dimensional receptor structures performed using MOE software running on a single computer Intel Pentium Dual Core. The algorithm used is the alpha sphere with a maximum RMS gradient convergence 0.01 kcal / mol Å and molecular mechanics force field parameters AMBER2
(b) *The Ligand file*: In a similar procedure, the ligand files were read in MOL, all hydrogens added, charges added and non-polar hydrogens merged and saved with .mol extension. Geometry optimization and energy minimization of three-dimensional structure fatty acid using ACD Lab software running on a single computer Intel Pentium Dual Core. The algorithm used is the alpha sphere with a maximum RMS gradient convergence 0.01 kcal / mol Å and molecular mechanics force field parameters MMFFx
(c) Preparing the docking parameter file: The docking parameter file, which instructs MOE about the ligand to move. The process begins with preparation docking files are done using a docking program contained in the MOE software. All the molecules fatty acid (to then called ligands) and the enzyme, hydrogen is added to both polar and charge while the hydrogen nonpolarnya MMFX in merge. File ligand and the enzyme is stored in the format Mole for later use in the preparation parameters. Docking calculation algorithm is run with the parameter Alpha sphere with population size 150, as many as 10 million energy evaluations and repetitions (search runs) as much as 100 times. This parameter is saved in Mdb format as a file that will be used to run the docking process.

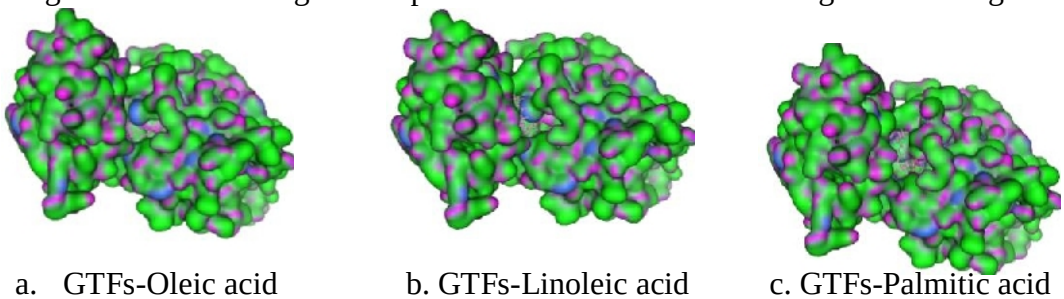
Results

The docking results of the fatty acid ligands result 3 best ligands. Screening based on ΔG and Lipinski's Rule of Five were employed from 3 ligands were resulted 1 of best ligand (Table 1).

Table 1. Docking results of GTFs and ligands

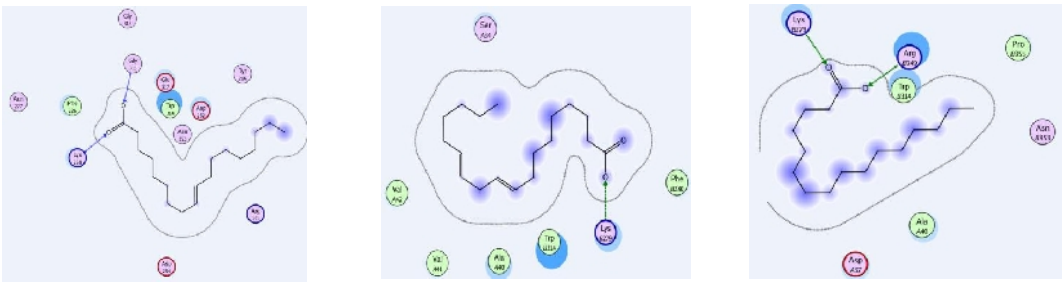
Ligands	ΔG (kcal/mol)	pKi (μM)	MR	H bonds	Log P
Linolenic acid	-3.4550	6.731	279.44	1	4.550
Linoleic acid	-4.2375	5.902	294.48	1	5.973
Oleic acid	-5.6863	7.262	281.460	2	4.774
Oleic metyl	-5.0030	5.447	296.495	-	6.197
Palmitic acid	-3.3276	6.155	255.422	2	4.218
Palmitic methyl	-3.7645	4.808	270.457	-	5.641

The three of best ligands have minimized ΔG and have high of pKi value.
The figure of three best ligandcomplexwith GTFs was shown at Figure 1 and Figure 2.



a. GTFs-Oleic acid b. GTFs-Linoleic acid c. GTFs-Palmitic acid

Figure 1.Complexs 3D of GTFs with Ligands



a. GTFs-Oleic acid b. GTFs-Linoleic acid c. GTFs-Palmitic acid

Figure 2. Complexs 2D of GTFs with Ligands

One of the best ligands were selected based on the number of hydrogen bond as the interaction to the catalytic site of GTFs (Table 2). High score and distance of hydrogen bond shown at Table 2

Tabel 2. Score and distance complex GTFs-Ligands of Hydrogen bond

Ligand	Hydrogen Bond	Score (%)	Distance (Å)
Linolenic acid	Lys279-O	55.2	2.51
Linoleic acid	Lys351-O	32.9	2.53
Oleic acid	Lys228-O	28.7	2.61
	Gly315-O	35.8	2.77
Oleic methyl	-	-	-
Palmitic acid	Lys229-O	14.8	2.42
	Arg349-O	30.9	2.49
Palmitic methyl	-	-	-

Discussion

The docking result of oleic acid has pKi 7.262 μM, this value indicate that the oleic acid has stronger affinity and interaction to form complex compounds with glucosyltransferases (GTFs) compare to another fatty acids. The value of pKi of oleic acid ligand is effective and interact strongly with GTFs (table 1). This means that the larger the value of the pKi, the smaller the Ki of the ligand. So the value of pKi can be used to determine the level of effectiveness in forming the complex enzyme with ligand.
Hydrophobic molecules show better log P (table 1). The ligand oleic acid (4.774), palmitic acid (4.218), and linoleic acid (5.973). Lipinski's rule of five mention that the poor absorption or permeation are more likely when the log P is over 5. Hydrophobic nature is shown green color, hydrogen binding is shown by cyan color and mid polar is shown by blue color (figure 1).

Hydrogen bonding of 3 best docking fatty acid was identified by MOE 2008.10 software on Lig X interaction program. The result of identification of hydrogen bonds between amino acid residues with GTFs ligand can be seen in table 2. Oleic acid has two H-bonds, linoleic acid has one H-bond while palmitic acid has two H-bonds. Hydrogen bonding contribute to the affinity of the ligand in forming the complex with GTF enzyme, which is due to electrostatic interaction between hydrogen atom of oxygen or hydrogen atom of ligand with a residues vice versa. Oleic acid ligand bind to catalytic site into the site of Lys228 and Gly315 which formed hydrogen bond with the active site of two bonds with score Lys (28.7 %) and H distance 2.61 (Å), Gly (35.8 %) and H distance 2.77 (Å). Linoleic acid bind to catalytic site into the site of Lys 351 with the active site of one bond with score (32.9 %) and H distance 2.53 (Å). Palmitic acid bind to catalytic site into the site of Lys 229 and Arg 349 at the active site of the two bonds with Lys (14.8 %) and Arg (30.9 %) and the H distance is 2.42 (Å) and 2.49 (Å).

Conclusion from this study we found the best ligand for docking glucosyltransferase is oleic acid and further study can be performed experiment in laboratory by using natural substances to inhibit glucan forming and prevent dental caries.

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