Molecular Docking Studies of Catechin and Its Derivatives as Anti-bacterial Inhibitor for Glucosamine-6-Phosphate Synthase

H Fikrika¹, L Ambarsari¹, T Sumaryada²,³,₄
¹Department of Biochemistry, Bogor Agricultural University, Jalan Agatis Kampus Dramaga IPB, 16880, Indonesia
²Computational Biophysics and Molecular Modeling Research Group (CBMoRG), Department of Physics, Bogor Agricultural University, Jalan Meranti Kampus IPB Dramaga Bogor 16680, Indonesia
³Biopharmaca Research Center, Bogor Agricultural University, Jalan Taman Kencana No. 3, Bogor 16128, Indonesia

E-mail: tsumaryada@ipb.ac.id

Abstract. Molecular docking simulation of catechin and its derivatives on Glucosamine-6-Phosphate Synthase (GlmS) has been performed in this research. GlmS inhibition by a particular ligand will suppress the production of bacterial cell wall and significantly reduce the population of invading bacteria. In this study, catechin derivatives i.e epicatechin, gallocatechin and epigallocatechin were found to have stronger binding affinities as compared to natural ligand of GlmS, Fructose-6-Phosphate (F6P). Those three ligands were docked on the same pocket in GlmS target as F6P, with 70% binding sites similarity. Based on the docking results, gallocatechin turns out to be the most potent ligand for anti-bacterial agent with ΔG= -8.00 kcal/mol. The docking between GlmS and catechin derivatives are characterized by a constant present of a strong hydrogen bond between functional group O3 and Ser-349. This hydrogen bond most likely plays a significant role in the docking mechanism and binding modes selection. The surprising result is catechin itself exhibited a quite strong binding with GlmS (ΔG= -7.80 kcal/mol), but docked on a completely different pocket compared to other ligands. This results suggest that catechin might still have a curing effect but with a completely different pathway and mechanism as compared to its derivatives.

1. Introduction
Tea is the most popular beverage in the world. One kind of tea is green tea, which has a high level of flavonoid compounds due to the absence of the oxidation process [1]. Those compounds, catechin and its derivatives (epicatechin, gallocatechin, and epigallocatechin) [2], have been known for a long time to have anti-oxidant, anti-diabetic, anti-bacterial, anti-inflammation, anti-virus, and anti-cancer potential [3].

The Glucosamine-6-phosphate synthase (GlmS) enzyme plays an important role in the synthesis of Bacterial cell wall. GlmS help the enzymatic reaction between fructose-6-phosphate (F6P) and L-glutamine to produce glucosamine-6-phosphate which lead to the formation of UDP-N acetylglucosamine which plays an important role in building peptidoglycan of bacterial cell wall. Inhibiting GlmS with a particular ligand will suppress the production of glucosamine-6-phosphate and kill the bacteria [4]. Inhibition of GlmS for anti-microbial purpose have been conducted by...
Balachandran et al [5]. In that paper N-benzyl-2,2,2 trifluoroacetamide was used as a ligand, and had been proven to be a potential anti-microbial drugs.

The potential of flavonoid compounds of green tea have been extensively studied by [6,7,8,9]. In this paper, the potential of catechin and its derivatives for anti-bacterial purposes were explored through a molecular docking method. Docking simulations were performed between catechin (and its derivatives) and GlmS enzyme as the target. Fructose-6-Phosphate (F6P), one of the natural inhibitors of GlmS, was used as the standard ligand. The docking of F6P to GlmS were important to the production of glucosamine-6-phosphate, which is crucial to the formation of bacterial cell wall. By comparing the inhibition profiles of catechin and its derivatives with F6P, we can define their potential as anti-bacterial agents.

2. Materials and Methods
There are three important parts in this research. The first part is preparation of target protein and ligands, the second is running the molecular docking simulation, and the last part is data analysis.

2.1. Receptor Preparation
Three dimensional structure of GlmS (pdb code 2VF5) was downloaded from www.rcsb.org (see figure 1) [10]. Receptor data were downloaded and opened using Discovery Studio Visualizer to remove the natural ligand that still attached to it. Before the docking process, the non polar hydrogen atoms were added, followed by Gasteiger charger calculation using Autodock tools (ADT) 1.5.6. [11]. The protein file then saved in pdbqt format and ready to be used for docking.

2.2. Ligand Preparation
There are five ligands prepared for docking simulation, one natural inhibitors as a control (or standard) and four catechin and its derivatives (as test ligands). Three dimensional structure of ligands were downloaded from PubChem (see figure 1), opened and saved in pdb format using Marvin View 6.0 program from ChemAxon (http://www.chemaxon.com). Polar hydrogen atoms were added and Gasteiger charges were assigned using Discovery Studio 3.5 Client. All ligands has to be saved in pdbqt format.

2.3. Docking Methods
AutoDock vina [12] program was used to dock ligand with GlmS. Targeted docking method was used in this research with the coordinate of origin was set at x=30.054, y=22.75 and z= 4.171. This set of numbers was based on the location of F6P binding with Glms from protein data bank file. The box size was set at x=40, y=40 and z=40. Docking simulation were run with the number of modes set to 20 to get more accurate results.

2.4. Docking Analysis
Analysis of binding affinity and the binding sites were performed in this research. LigPlot 1.4.5 [13] was used to produce two dimensional docking representations of all ligands. There are two types of interaction analyzed, hydrogen bond and hydrophobic interaction. Interaction occurred between amino acid residue on the target side and functional group on the ligand side. Binding site similarity analysis between test ligands and standard (natural) ligand were performed in this research to show the potential of catechin and its derivatives as anti-bacterial agent.

3. Results and Discussions
The docking simulation results are shown in Table.1. F6P as standard ligand binds with GlmS with binding affinity of -6.00 kcal/mol (see figure.2). The binding sites of F6P on GlmS target includes five residues involved in the hydrogen bond and six residues involved in hydrophobic interactions. The strongest binding occurred at Ser-303 in which two hydrogen bonds were formed with functional group O3 (bond length of 2.89 Å ) and O1(bond length of 3.24 Å).
Table 1 shows that catechin were docked at completely different sites as compared to other ligands, as can be seen from zero binding site similarity. The binding affinity of catechin on GlmS is found to be -7.80 kcal/mol. This strong binding is mostly due to strong hydrogen bonds between Val-567 with functional group O5 (bond length of 2.72 Å) and O6 (bond length of 2.75 Å) as seen in figure 2. There are four residues involved in hydrogen bond and nine in hydrophobic interactions.

Epicatechin docked on the same region as F6P. Functional group O3 made two hydrogen bonds with Ser-348 and Ser-349. Ten hydrophobic interactions surround epicatechin inside the binding pocket (figure 3). The binding site similarity of epicatechin to F6P is 70.0% (7 out of 10 residues). The binding site similarity of all ligands as compared to F6P is also shown by highlighted residues in Table 1. The binding affinity of epicatechin is -7.50 kcal/mol, with the strongest interaction found in hydrogen bond between O3 and Gln-348. Two residues, Thr-352 and Val-399, that previously involved in hydrogen bond with F6P, now changed into hydrophobic interactions with epicatechin.

Gallocatechin has the strongest binding affinity as compared to other ligands with ∆G= -8.00 kcal/mol. This strong binding is due to the existence of four hydrogen bonds, with three of them have bond length < 3.00 Å. There are eight GlmS residues involved in hydrophobic interactions, as seen in figure 3. Two residues, Thr-352 and Glu-488, that previously involved in hydrogen bond with F6P now switched into hydrophobic interactions with gallocatechin. The binding site similarity of gallocatechin as compared to F6P is 70% (7 similar residues out of 10 GlmS residues docked by F6P).

Epigallocatechin has the binding affinity of -7.90 kcal/mol with similar type of hydrogen bond as epicatechin has (Gln-348 and Ser-349 hydrogen bond with functional group O3). One more hydrogen bond is also formed between Gln-348 with functional group O2. Ten hydrophobic interactions with identical sites as compared to epicatechin surround epigallocatechin (figure 3). The binding site similarity of epigallocatechin as compared to F6P is also 70%. Gln-348 which previously involved in hydrophobic interaction with F6P now changed into hydrogen bond with epigallocatechin.

Ser-349 is found to be a continuously present hydrogen bond in F6P, epicatechin, gallocatechin and epigallocatechin. In catechin derivatives, all hydrogen bonding with Ser-349 is occurred in functional group O3. Interaction between functional group O3 in catechin derivatives and Ser-349

---

**Figure 1.** Structure of target protein GlmS (pdb code 2PV5) and test ligands. Protein structure downloaded from www.rcsb.org and ligand’s structures were downloaded from http://pubchem.ncbi.nlm.nih.gov
residue in GlmS seems important and play a crucial role in determining the potential of catechin derivatives as anti-bacterial agent. The existence of Glu-488 binding site in the docking of F6P and catechin derivatives is in agreement with the results from Balachandran et.al [5] that used N-benzyl-2,2,2 trifluoroacetamide ligand for anti-microbial purpose. This finding emphasizes the results of our docking simulation is on the right track in searching for anti-bacterial and anti-microbial agents.

Table 1. Docking simulation results

| Test ligand | Hydrogen bond length (Å) | Residue involved in H-bonding | Functional group of ligand | Residue involved in Hydrophobic interaction | Number of binding site similarity to standard ligand | Binding Affinity ΔG (kcal/mol) |
|-------------|--------------------------|-------------------------------|---------------------------|------------------------------------------|---------------------------------------------|-------------------------------|
| Fructose-6-Phosphate (standard ligand) | 2.89 | Ser-303 | O3 | Thr-302, Ser-347, Gln-348, Ala-400, Ser-401, Ala-602 | All | -6.00 |
| | 3.24 | Ser-303 | O1 | Gln-488, Ala-400, Ser-401, Ala-602 | | |
| | 3.22 | Ser-349 | O2 | | | |
| | 3.18 | Thr-352 | O9 | | | |
| | 3.02 | Val-399 | O8 | | | |
| | 3.09 | Glu-488 | O8 | | | |
| Catechin | 3.16 | Arg-472 | O3 | Ala-520, Pro-521, Leu-525, Asp-548, His-566, Glu-569, Ala-572, Pro-573, Tyr-576 | 0 | -7.80 |
| | 2.93 | Asp-474 | O3 | Ala-520, Pro-521, Leu-525, Asp-548, His-566, Glu-569, Ala-572, Pro-573, Tyr-576 | | |
| | 3.23 | Asn-522 | O4 | | | |
| | 2.72 | Val-567 | O5 | | | |
| | 2.75 | Val-567 | O6 | Kg | | |
| | 3.25 | Val-567 | O6 | Kg | | |
| Catechin | 3.16 | Gln-348 | O3 | Cys-300, Gly-301, Ser-347, Thr-352, Val-399, Leu-484, Glu-488, Leu-601, Ala-602, Lys-603 | 7 | -7.50 |
| | 3.09 | Ser-349 | O3 | Cys-300, Gly-301, Ser-347, Thr-352, Val-399, Leu-484, Glu-488, Leu-601, Ala-602, Lys-603 | | |
| Galloallocatechin | 2.80 | Thr-302 | O5 | Cys-300, Gly-301, Ser-347, Thr-352, Val-399, Leu-484, Glu-488, Leu-601, Ala-602, Lys-603, Ser-604 | 7 | -8.00 |
| | 2.93 | Gln-348 | O3 | Cys-300, Gly-301, Ser-347, Thr-352, Val-399, Leu-484, Glu-488, Leu-601, Ala-602, Lys-603, Ser-604 | | |
| | 2.92 | Ser-349 | O3 | Cys-300, Gly-301, Ser-347, Thr-352, Val-399, Leu-484, Glu-488, Leu-601, Ala-602, Lys-603, Ser-604 | | |
| | 3.11 | Ala-602 | O7 | Cys-300, Gly-301, Ser-347, Thr-352, Val-399, Leu-484, Glu-488, Leu-601, Ala-602, Lys-603, Ser-604 | | |
| Epigallocatechin | 3.15 | Gln-348 | O2 | Cys-300, Gly-301, Ser-347, Thr-352, Val-399, Leu-484, Glu-488, Leu-601, Ala-602, Lys-603, Ser-604 | 7 | -7.90 |
| | 3.00 | Gln-348 | O3 | Cys-300, Gly-301, Ser-347, Thr-352, Val-399, Leu-484, Glu-488, Leu-601, Ala-602, Lys-603, Ser-604 | | |
| | 3.08 | Ser-349 | O3 | Cys-300, Gly-301, Ser-347, Thr-352, Val-399, Leu-484, Glu-488, Leu-601, Ala-602, Lys-603, Ser-604 | | |
Figure 2. Docking representation of catechin on GlmS target.

Figure 3. Comparison of docking (binding) sites of (a) Fructose-6-Phosphate, (b) gallocatechin, (c) epigallocatechin and (d) epicatechin on GlmS target. Note that all ligands docked on almost identical region (pocket).
4. Conclusions
Molecular docking simulation of catechin and its derivatives on GlmS target has been performed. All catechin derivative ligands exhibited a strong binding with GlmS with gallocatechin shows the best potential to be developed into an anti-bacterial drug. Except for catechin, all ligands in principal docked on the same pocket (binding sites) which indicates the same biochemistry pathway as compared to F6P, the natural ligand of GlmS. Catechin, on the other hand has also shown a strong binding to GlmS, but docked on a completely different pocket as compared to F6P. This result arise a new question whether catechin still have the remedy effect, but following a different pathway and mechanism as compared to F6P. Further study is clearly needed here.

5. Acknowledgment
The authors express their gratitude for the funding from the Directorate of Higher Education, Ministry of Education and Culture Republic of Indonesia through BOPTN research grant contract number 237/IT3.41.2/L2/SPK/2013 code 2013.089.521219.

6. References
[1] Banerjee S and Chatterjee J. 2014 J.Food Sci.Technol 487-3
[2] Gramza A, Korczak J and Amarowicz R. 2005 A Review. Pol. J. Food Nutr. Sci. 14(3) 219-235
[3] Dubreuil J D 2013 Toxins. 5 2009-2041
[4] H Chmara, E Borowski 1986 Acta. Microbiol. Pol 35 15-27
[5] Balachandran C et al 2015 Appl.Nanosci 5 207-216
[6] Kumar D et al 2015 J. Korean Soc Appl.Biol.Chem 58(4) 581-585
[7] Reygaert W C 2014 Frontiers in Microbiology 5 (434) 1-8
[8] Taylor P W, Hamilton-Miller J M T, Stapleton P D. 2005 Food.Sci.Technol.Bull 2 71-81
[9] Lee L S, Kim S H, Kim Y B and Kim Y C 2014 Molecules 19 9173-9186
[10] Morriell S, Badet D M A, Golinell P B 2014 J Mol Biol. 337:1174
[11] Morris G M, Huey R, Lindstrom W, Sanner M F, Belew R K, Goodsell D S and Olson A J 2009 J. Computational Chemistry 16 85-91
[12] Trott O, Olson A J, 2010 Journal of Computational Chemistry 31 455-461
[13] Laskowski R A, Swindells M B 2011 J Chem.Inf.Model. 51(10) 2778-2786