Molecular investigation of modified β-cyclodextrin and cholesterol inclusion complexes through molecular docking simulations

M A F Nasution, H G Riyanto, E Saepudin and T A Ivandini
Department of Chemistry, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Indonesia, Depok 16424, Indonesia

Corresponding author’s email: ivandini.tri@sci.ui.ac.id

Abstract. Hypertension, a medical condition which commonly associated with cardiovascular diseases, the most lethal non-communicable diseases in the world, is indicated by the high blood cholesterol level. Thus, maintaining the blood cholesterol level is essential especially for hypertension-diagnosed patients. In this study, the molecular docking simulations were successfully performed between cholesterol and methylene blue (MB) with the modified β-cyclodextrin (BCD) compounds as the guest and host molecules, respectively, to investigate their molecular interaction when forming the inclusion complexes. The docking results showed that the modification on the -OH hydroxyl group at position 6 of BCD improves the binding affinity of the cholesterol when forming the inclusion complex, where the -OCH₃ modification has the highest binding affinity toward cholesterol, with ΔGbinding value of -5.9 kcal/mol, followed by -OCH₂CHO, -OCH₂COOH and -OCOCOH(COOH)₂, with ΔGbinding value of -5.8 kcal/mol. Moreover, the hydrophobic and van der Waals interactions were observed as the most dominant interactions when both BCD-modified compounds/cholesterol and BCD-modified compounds/MB inclusion complexes were formed. Thus, the electrochemical technique can be employed toward the cholesterol and these BCD-modified compounds to validate the docking results and determine its sensitivity as cholesterol sensor.

Keywords: BCD, cholesterol, non-enzymatic sensor, molecular docking simulation.

1. Introduction
Cardiovascular diseases (CVDs) remain the highest cause of death in the world, with approximately 16 million deaths caused by this disease [1]. In Indonesia itself, this non-communicable diseases rank is at first and second position as the leading cause of death around the archipelago, which according to the latest statistics, about 6.52 million and 5.18 million people died each year because of ischaemic heart disease and cerebrovascular disease, respectively [2]. To date, hypertension is still regarded as the leading cause of CVDs, which is indicated by the irregularly high level of blood pressure, both systolic and diastolic (≥ 140 mmHg and ≥ 90 mmHg, respectively), due to the plaque buildup in the blood vessels [3, 4]. In a long term, without proper treatment, the plaque will thicken the blood vessels, disturb the blood circulation, and eventually trigger angina pectoris [5]. Recent studies show that high cholesterol concentration in the blood is directly associated with the higher CVDs risk [6]. Therefore,
maintaining the blood cholesterol level is essential, especially for someone who already diagnosed with hypertension to avoid having CVDs risk in the future.

β-cyclodextrin (BCD) is a cyclic oligosaccharide which consisted of seven glucopyranose units that has a hydrophobic region inside its cavity, and hydrophilic interior which is formed by its primary and secondary -OH groups [7]. Due to its unique molecular shape and structure, BCD can entrap a molecule within its hydrophobic cavity, formed an inclusion complex that can be utilized for several applications, such as drug carriers for hydrophobic drugs in the drug delivery system [8]. Recently, BCD-based material has also been widely utilized in the electrochemical sensing field to detect several biomolecules [9, 10]. Moreover, BCD can also be easily modified in its primary -OH groups without removing its entrapment ability by substituting its polar hydrogen atom with citrate or carboxymethyl functional group, enhancing its hydrophilicity for the drug delivery purposes [11, 12]. However, the effect of this modification on BCD for its sensor application has not been fully understood yet. Since both BCD and cholesterol do not produce electrochemical signals that are needed for this complex to be functioned as a sensor using electrochemical method, methylene blue (MB), a dye-based heterocyclic aromatic from thiazine group, can be applied as redox indicator that can undergo redox reactions involving two electrons and one proton in the reduced form of leucomethylene blue (LMB) at physiological pH [13].

In this research, the computational study using molecular docking simulation was employed to predict the binding affinity and conformation of the formed inclusion complex of both cholesterol and MB with BCD-modified compounds. Therefore, the result of this study can be used as the reference before the synthesis, characterization, and application of the BCD-modified compounds as the cholesterol sensor took place.

2. Methodology

The methodology of this research was conducted based on the modification of the previous research [14] using various software that has been widely used and validated through a series of experiments [15, 16]. At first, the BCD structure was obtained from the RCSB-PDB website with PDB ID: 3CK8 [17], followed by the structure optimization by removing the SusD protein and other unnecessary molecules such as ethylene glycol and water, while keeping the BCD structure using BIOVIA Discovery Studio 2019 software. The modification of BCD was took place on the primary -OH group at position 6 using BIOVIA Discovery Studio 2019 software as well (figure 1).

Furthermore, the cholesterol and MB structures were obtained from the PubChem website [18] with Pubchem CID: 5997 and 4139, respectively, and their 3D structure were retrieved in .sdf files. These structures were then prepared and optimized using default minimization protocol in PyRx v0.8.0 software [16], with MMFF94x was selected as the forcefield.

\[
\begin{align*}
R_0 &= -\text{OH} \\
R_1 &= -\text{OCH}_3 \\
R_2 &= -\text{NH}_2 \\
R_3 &= -\text{OCH}_2\text{CHO} \\
R_4 &= -\text{OCH}_2\text{COOH} \\
R_5 &= -\text{OCOCH}((\text{COOH})_2
\end{align*}
\]

**Figure 1.** The substituted functional group at primary -OH position 6 that used in this study.
The minimized structure was then saved and converted into .pdbqt file format for further use. After that, the standard protocol with adjusted parameters of molecular docking simulation of BCD and BCD-modified compounds against cholesterol and MB was performed using AutoDock Vina module in PyRx v0.8.0 software. Finally, the docking results were then observed and the conformations that have the highest binding affinity of each compound against their respective receptors were visualized using BIOVIA Discovery Studio 2019 software.

3. Results and discussion

According to the previous study, the molecular interaction between BCD and its guest molecule in the hydrophobic cavity are predominately based on hydrophobic interaction, electrostatic interaction, van der Waals interaction, and hydrogen bonds [19]. Thus, the conformation of BCD-cholesterol and BCD-MB complex inclusions can be predicted using molecular docking simulations. To be functionalized as a cholesterol sensor using electrochemical methods, the MB must be included in the sensor system as the electroactive species that can give electrochemical signals, unlike cholesterol and BCD. Moreover, MB was also chosen as a redox indicator because it has a good selectivity for cholesterol even in the presence of other biomolecules that can be potentially acted as interference compounds that can disturb the cholesterol measurements [13]. As depicted in figure 2, the mechanism of BCD-cholesterol inclusion complex formation from BCD-MB complex can be explained as follows: the BCD that has been immersed and dispersed first with MB solution is dissolved with a cholesterol solution. Since cholesterol has more hydrophobicity than MB due to its bulky steroid structure and hydrocarbon tail, it will substitute MB from the hydrophobic cavity of BCD, forming BCD-cholesterol inclusion complex that has a higher binding affinity and more stable complex than its counterpart. The release of MB from BCD will increase the concentration of MB in the solution, which is then assumed to be exact concentration of the added cholesterol in the system. Through electrochemical techniques, such as voltammetry and amperometry, the value of both oxidized and reduced MB can be observed and analyzed to determine the cholesterol level contained in the solution.

In this study, the modification has taken place at the primary -OH hydroxyl group at position 6, this was done because in theory, this -OH group is the most basic, least acidic, and most accessible -OH group among all, which can be easily attacked by electrophilic agent after all the -OH groups are deprotonated using a strong, excess base reagent [20]. For this study, about six BCD-based compounds were drawn and formed, including the unmodified BCD. The other five modified BCDs have their primary -OH hydroxyl group at position 6 substituted with another functional group, namely methoxy...
(-OMe/-OCH₃), amine (-NH₂), formylmethoxy (-OCH₂CHO), carboxymethoxy (-OCH₂COOH), and dihydrogen citrate (-OCOCOH(COOH)₂). These functional groups were chosen since they have already been studied and successfully synthesized on BCD in the previous research [12, 20]. These compounds were computationally prepared and optimized using default parameters that have been extensively used and validated for the docking simulations [14]. The docking results in this study can be seen in table 1.

According to the docking results that have been performed, it was determined that the -OCH₃ modification at BCD gave the highest binding affinity toward cholesterol, compared to the other five BCD-based compounds. The formation of BCD-OCH₃ modified compound and cholesterol inclusion complex has ΔGbinding value of -5.9 kcal/mol and pKeq value of 4.3434, this observed ΔGbinding and pKeq value were respectively lower and higher than the BCD-OCH₂CHO, BCD-OCH₂COOH, and BCD-OCOCOH(COOH)₂ modified compounds, which were observed at -5.8 kcal/mol and 4.2698, respectively. Finally, BCD-NH₂ modified compound has the highest observed ΔGbinding value toward cholesterol, which determined at -5.7 kcal/mol with pKeq value of 4.1962. Nonetheless, these five BCD-modified compounds have lower ΔGbinding values than the unmodified BCD, which were determined at -5.4 kcal/mol (pKeq value observed at 3.9753). This phenomenon indicated that the modification of primary -OH hydroxy group at position 6 did affect the increase binding affinity of the cholesterol inclusion complexes, mainly due to the increasing number of atoms in the BCD, which contributes to the hydrophobic and van der Waals interactions in the hydrophobic cavity of BCD. Moreover, the docking simulation also revealed that cholesterol has a higher binding affinity toward any BCD-modified compound than MB, where the difference was ranged between -0.1 kcal/mol (at -NH₂) to -0.9 kcal/mol (at -OCH₃). This also indicated that the presence of cholesterol would eventually replace MB in the hydrophobic cavity of BCD, since the formation of BCD-cholesterol inclusion complex is thermodynamically favorable compared to the BCD-MB complex, according to the docking results.

The molecular interactions of cholesterol and MB with BCD and its modified compounds were also observed after the docking simulations were performed. As depicted in figure 3, the interactions between the BCDs with their guest molecules were primarily dominated by the hydrophobic and van der Waals interactions, although π-hydrogen bonds may occasionally occur in the BCD-MB inclusion complex between the aromatic ring of MB and polar hydrogen atoms from BCD. Moreover, according to the docking results, it was also observed that when forming the BCD-cholesterol inclusion complex, the hydrocarbon tail of cholesterol was exposed out on the wider edge of BCD cavity, where the secondary -OHs are located, while the polar hydroxyl and bulky steroid groups of cholesterol were positioned on the narrow edge and hydrophobic cavity of BCD, respectively.

These results were in accordance with the docking results from the previous study [21]. Although the different conformation of BCD-cholesterol inclusion complex was observed as well (polar hydroxyl group and hydrocarbon tail of cholesterol were located on wider and narrow edges, respectively),

| -R          | ΔGbinding | pKeq   | ΔGbinding | pKeq   |
|-------------|-----------|--------|-----------|--------|
| -OH         | -5.4 kcal/mol | 3.9753 | -5.3 kcal/mol | 3.9017 |
| -OCH₃       | -5.9 kcal/mol | 4.3434 | -5.0 kcal/mol | 3.6809 |
| -NH₂        | -5.7 kcal/mol | 4.1962 | -5.6 kcal/mol | 4.1226 |
| -OCH₂CHO    | -5.8 kcal/mol | 4.2698 | -5.5 kcal/mol | 4.0490 |
| -OCH₂COOH   | -5.8 kcal/mol | 4.2698 | -5.6 kcal/mol | 4.1226 |
| -OCOCOH(COOH)₂ | -5.8 kcal/mol | 4.2698 | -5.5 kcal/mol | 4.0490 |
but since it has a higher $\Delta G_{\text{binding}}$ and lower $\text{pK}_{\text{eq}}$ value than the former, the formation of these complex was not likely to be happened due to it was not thermodynamically favorable compared to its counterparts.

Figure 3. Molecular interaction of inclusion complex of BCD-modified compound (a) $R = \text{-OMe}$, (b) $\text{-CH}_2\text{COOH}$ and (c) $\text{-OCOCOH(COOH)}_2$ with cholesterol (left) and methylene blue (right).
4. Conclusion
In this study, a series of molecular docking simulations of cholesterol and MB against BCD and its modified compounds were successfully performed. In summary, the modification of BCD in the primary -OH at position 6 did increase the binding affinity of the cholesterol, through some modification also decreasing the binding affinity of MB as well. According to the docking results, the -OCH₃ modification has the highest binding affinity toward cholesterol, with $\Delta G_{\text{binding}}$ value of -5.9 kcal/mol, followed by -OCH₂CHO, -OCH₂COOH, and -OCOCOH(COOH)$_2$, with $\Delta G_{\text{binding}}$ value of -5.8 kcal/mol. Thus, these compounds can be later synthesized and characterized before the electrochemical application as a cholesterol sensor for these BCD-modified compounds can be performed. Furthermore, the molecular dynamics studies can be carried out as well in the future to know the exact molecular mechanism of the formation of BCD-modified and cholesterol/MB inclusion complexes.

Acknowledgments
This work is financially supported by the Directorate of Research and Community Engagement, Universitas Indonesia, through Hibah Penelitian Unggulan Perguruan Tinggi (PUPT) Tahun Anggaran 2019 No: NKB-1599/UN2.R3.1/HKP.05.00/2019. No conflicts of interest are declared regarding this research and publication.

References
[1] WHO 2017 Cardiovascular Diseases (CVDs) available at https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)
[2] Mboi N et al. 2018 Lancet 392 581-91
[3] Balakumar P, Maung-U K and Jagadeesh G 2016 Pharmacol. Res. 113 600-9
[4] Goldstein J L and Brown M S 2015 Cell 161 161-72
[5] Van Deventer H E et al. 2011 Clin. Chem. 57 490-501
[6] Deedwania P C, Carbajal E V and Bobba V R 2007 Clin. Cardiol. 30 (Suppl. I) I16-24
[7] Jambhekar S S and Breen P 2016 Drug Discov. Today 21 356-62
[8] Loftsson T 2002 J. Incl. Phenom. Macro. Chem. 44 63-7
[9] Huang J et al. 2012 Anal. Methods 4 4264-8
[10] Alarcón-Angeles G et al. 2008 Carbon 46 898-906
[11] Chen P, Yao S, Chen X, Huang Y and Song H 2019 New J. Chem. 43 4282-90
[12] Jayaprabha K N and Joy P A 2015 RSC Adv. 5 22117-25
[13] Arvand M, Sohrabnezhad S, Mousavi M F, Shamsipur M and Zanjanchi M A 2003 Anal. Chim. Acta 491 193-201
[14] Tambunan U S F, Parikesit A A, Wardani F, Nasution M A F and Kerami D 2017 Rasayan J. Chem. 10 910-21
[15] BIOVIA D S 2016 Discovery Studio Modeling Environment, Release 2017, San Diego Dassault Systèmes available at https://www.3dsbiovia.com/products/collaborative-science/biovia-discovery-studio
[16] Dallakayan S and Olson A J 2015 Chem. Biol. 1263 243-50
[17] Koropatkin N M, Martens E C, Gordon J I and Smith T J 2008 Structure 16 1105-15
[18] Kim S et al. 2016 Nucleic Acids Res. 44 D1202-13
[19] Inoue Y, Liu Y, Tong L H, Shen B J and Jin D Sen 1993 J. Am. Chem. Soc. 115 10637-44
[20] Řezanka M 2016 Eur. J. Org. Chem. 2016 5322-34
[21] Choi Y-H, Yang C-H, Kim H-W and Jung S 2001 J. Incl. Phenom. Macro. Chem. 39 71-6