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UNDERSTANDING THE INTERACTION BETWEEN GLUTATHIONE AND ACETAMINOPHEN: A DOCKING STUDY APPROACH

Mohammad Rizki Fadhil Pratama, Eko Suhartono
Universitas Muhammadiyah Palangkaraya, RTA Milono St. Km.1,5, Palangka Raya
Biochemistry and Biomolecular Department, Medical Faculty, Lambung Mangkurat University, Banjarbaru

ABSTRACT

Background:*Acetaminophen (PCT) is known for its pro-oxidant properties, which neutralized by the presence of internal antioxidants such as glutathione (GSH). GSH has two forms: monomers and dimers, mainly distinguished by the presence of thiol group.* ***Purpose:****This study aims to see the difference in interaction between PCT with both forms of GSH.* ***Method:****Molecular docking was performed using Autodock Vina 1.1.2 on whole GSH surfaces. The main parameter used was the free energy of binding as affinity marker, as well as the position of PCT toward GSH.* ***Result:****The docking results show that PCT has a slightly higher affinity to the dimeric form of GSH than its monomeric form with the free energy of binding -2.7 kcal/mol and -2.2 kcal/mol, respectively. The interesting thing is the acetyl group of PCT is in a position far from the thiol group in the monomeric form of GSH, in contrast to its dimeric form.* ***Conclusion:****These results show that difference in affinity of PCT to both GSH forms is influenced by the position of the acetyl group against the thiol group of cysteine in GSH. The proximity of the pro-oxidant group to the thiol group leads to an increase in the affinity of the pro-oxidant to GSH.*

Keywords*: Acetaminophen, Docking, Glutathione*
Corespondence address :Mohammad Rizki Fadhil Pratama, Universitas Muhammadiyah Palangkaraya, RTA Milono St. Km.1,5, Palangka Raya, Indonesia; e-mail: m.rizkifadhil@umpalangkaraya.ac.id

INTRODUCTION

Acetaminophen (PCT)is a synthetic analgesic that is often used by dentists to reduce pain in teeth.PCT could be considered the drug of choice for pain relief because it interferes less within tooth movement[1]. PCTstill has a deficiency in terms of toxicity where the hepatotoxic nature of PCT is often a constraint in terms of administration. Those hepatotoxic properties are mainly influenced by the pro-oxidant properties of the PCT's active group, which unfortunately,still not possible to be substituted with other active groups[2].

The mechanism of action of PCT involving the redox mechanism shows the dependence on the presence of pro-oxidant groups such as phenol[3]. Extensive oxidative stress induced by PCT involves cytochrome P450 and mitochondrial pathway, which is difficult to avoid because of the nature of the phenol group[4].This characteristic is a problem especially in developing countries, where PCT side effects are one of the main causes of acute hepatic failure[5].

Various studies to find ways to overcome these toxic properties have produced solutions using various antioxidant ingredients[6].Some external antioxidant compounds are known to reduce and even neutralize the hepatotoxic properties of PCT, such as curcumin[7]. The use of curcumin tablets along with PCT is known to reduce hepatotoxic cases[8]. However, several studies show that the use of curcumin can also cause serious side effects even though further research is still needed[9,10].

Besides external antioxidants, our bodies are also endowed with internal antioxidants in the form of enzymes and small molecular weight compounds[11]. Glutathione (GSH), a small molecul compound±307 Da is the most abundant antioxidantaerobic cells, presenting with high concentrations in body fluidsand tissue. GSH is synthesized from L-glutamate, L-cysteineand L-glycine. GSH is very important to protect tissuesoxidative stress, acting as free radical scavengers and lipid peroxidation inhibitors[12]. GSH is one of the main internal antioxidants that protects

cells from the pro-oxidant properties of PCT, mainly because of the presence of thiol groups in GSH which are the main scavenger for capturing free radicals caused by PCT[13,14]. Thiol groups are mainly owned by a variety of small molecule external antioxidants, in contrast to disulfide groups which are generally owned by larger antioxidants[15].

In general, GSH has two forms, namely monomer and dimer[16-17]. Both forms of GSH have differences characteristic, where GSH monomers have thiol groups and its dimers have disulfide groups[18]. The difference in functional groups makes these two forms of GSH have different activity as antioxidants, including when interacting with pro-oxidant compounds such as PCT. In this research, molecular docking will be conducted to analyze the different types of interaction and affinity between the two forms of GSH toward PCT.Observation of thiol groups from GSH is mainly carried out to determine differences in antioxidant mechanisms of GSH.

MATERIALS AND METHODS

Preparation of Ligands

The ligand used was PCT as shown in Figure 1. Structure of PCT were sketched using GaussView 3.08 Software from Gaussian, Inc. Structure were geometry optimized by Hartree-Fock method basis set 3-21G with Gaussian 03W software from Gaussian, Inc. Geometry optimization was performed primarily to provide an ideal conformation that approaching conformation of these compound in nature[19,20]. Optimized structure format changed from .log to .pdb using OpenBabel 2.4.1 software so that it can be run by the docking software used[21-22]. Docking software used in this study was AutodockVina 1.1.2 from The Scripps Research Institute. Compared to autodock 4, Vina provides a better calculation rate with a higher level of accuracy[23]. However, Vina does not provide dissociation constant parameters as shown in Autodock 4[19,24]. Ligand then is given the charge and set torque using AutoDockTools 1.5.6 software from The Scripps Research Institute[25].

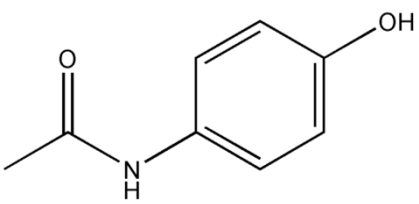

Figure 1.Two-dimensional structure of PCT

Preparation of Receptors

In this study the structure of proteins was not used as receptors but rather the molecules that become the interaction targets of the ligand. The receptors used was both two form of monomer and dimer GSHas shown in Figure2 and Figure 3. The structures of both receptors were sketched using GaussView 3.08 Software. The structures also weregeometry optimized by Hartree-Fock method basis set 3-21G with Gaussian 03W software.Optimized structure format then changed from .log to .pdb using OpenBabel 2.4.1 software. The receptors then added the non-polar hydrogen group, given the charge, and set the grid box and coordinate using AutoDockTools 1.5.6 software[22,25].

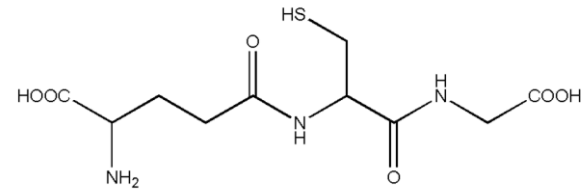

Figure 2. Two-dimensional structure of GSH monomer

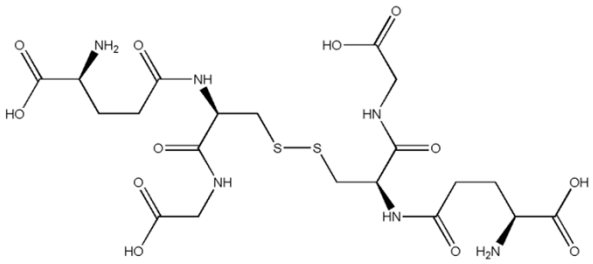

Figure 3. Two-dimensional structure of GSH dimer

Molecular Docking

Docking for the ligand performed using blind docking method, where entire the receptor surface was used as grid box area [26,27]. The main parameter used in docking process was the free energy of binding (ΔG) as affinity marker[28]. Ligand affinity to the receptor in docking method is determined by ΔG scores. The more negative ΔG indicated higher ligand affinity toward active site of the used receptor [29]. The position of ligand for each receptor then compared to assess the difference of interaction between ligand and both receptors[30].

RESULTS

Docking results was performed on the entire surface of each receptors with energy range, exhaustiveness, and number of modes parameters score 3, 8, and 9, respectively. These number is default parameter for Autodock Vina 1.1.2 for molecular docking. Higher parameter will increase the accuracy of docking results[23]. Docking results of PCT towards both receptor was shown in Table 2.

Table 1. Docking results of PCT towards monomer and dimer GSH

Parameter	Monomer GSH	Dimer GSH
ΔG(kcal/mol)	-2.2	-2.7

This study also shows the interaction between PCT and GSH monomers as well as dimers (Figures 3 and 4).

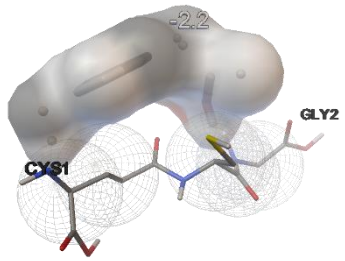

Figure 4. PCT position towards monomer GSH, thiol group is shown by yellow stick

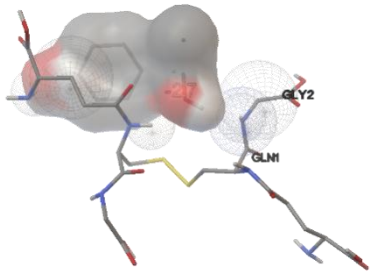

Figure 5. PCT position towards dimer GSH, disulfide bond is shown by yellow stick

DISCUSSION

The ligand show negative ΔG scores which indicating the interaction between PCT monomer and dimer GSH will occur spontaneously[31]the difference of ΔG scores from both receptor was substantial enough to show that PCT has a greater affinity for dimer GSH than its monomer. However, the difference is not significant enough as the difference of ΔG scores of 0.5 kcal/mol only gives the difference of dissociation constant score of 0.08264 μM[32]. That small difference in dissociation constant does not have a significant effect on the affinity of PCT on both forms of GSH[33], mainly because of the huge number of GSH molecules in the body.

Another important parameter to observe is the difference in position orientation of the PCT functional group to the thiol/disulfide group of each GSH forms, as can be seen in Figure 4 and 5. The interesting thing to observe is that the position of the acetyl group from PCT is very significant in both GSH types. In Figure 4, the acetyl group of PCT was located far from thiol group, whereas the acetyl group is the main functional group in PCT which contributes greatly to the pro-oxidant properties of PCT[34]. In contrast to dimer GSH, acetyl group of PCT was located very near to its disulfide bond, as shown in Figure 5. In fact, the steric resistance of the dimer GSH molecule is greater than its monomer form. The difference, although trivial, turns out to have an impact on the affinity of PCT on both forms of GSH. The likelihood of greater PCT affinity for dimer GSH than its monomer was due to the position of the acetyl group of PCT which is closer to the thiol/disulfide group on dimer GSH than its monomer form.

CONCLUSION

The present study succeeded in reveals the big difference of the interaction between PCT and both forms of GSH, which is mainly attributed by the difference in the presence of the main functional groups of each GSH. The difference in affinity between the two forms of GSH although substantial but not too significant.Eventually, it can be concluded that both monomers and dimers of GSH can interact with PCT to eliminate the pro-oxidant properties of PCT.The closer the pro-oxidant group leads to the thiol/disulfide group of GSH, the greater the affinity of GSH to the pro-oxidant compound. In other words, GSH will provide the most optimal antioxidant activity when the pro-oxidant group binds to the closest position to the thiol/disulfide group.This knowledge provides a new dimension in the development of antioxidant, especially those derived from synthetic compounds.

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