ABSTRACT

Introduction: Advanced glycation end products (AGEs) contribute to the pathogenesis of chronic inflammation, diabetes, micro and macrovascular complications, and neurodegenerative diseases through the binding with RAGE. Natural compounds can act as an alternative in disease therapy related to the AGEs-RAGE interactions. Cyanidin-3-O-glucoside is one of the potential anthocyanins found in red rice. Cyanidin-3-O-glucoside in red rice may interfere with the AGEs-RAGE signaling so that the potential mechanism of their interaction needs to be elucidated. Aim: This study aimed to investigate the potency of cyanidin-3-O-glucoside in red rice as an inhibitor of AGE-RAGE signaling pathway through in silico analysis. Methods: Our study used the 3D structures of AGEs and Cyanidin-3-O-glucoside compounds from PubChem and Receptor for AGEs (RAGE) from the RCSB Protein Data Bank (PDB) database. The molecular interactions of those compounds and RAGE were established using Hex 8.0 software, then visualized using Discovery Studio 2016 software. Results: Argypirimidine, pentosidine, pyrralline, and imidazole bound to the ligand-binding domain of RAGE with the binding energy of -247 kcal/mol, -350.4 kcal/mol, -591.1 kcal/mol, and -100.4 kcal/mol, respectively. The presence of cyanidin-3-O-glucoside in the imidazole-RAGE-cyanidin-3-O-glucoside complex could inhibit the interaction of imidazole-RAGE with a binding energy of -299 kcal/mol, which was lower than of imidazole-RAGE complex. The establishment of AGEs-Cyanidin-3-O-glucoside-RAGE complex showed that cyanidin-3-O-glucoside, which bound first to Argypirimidine and Pyrralline, could bind to RAGE at the same residue as those two AGEs did with the binding energy of -411.8 kcal/mol and -1305 kcal/mol, respectively. Based on the binding site location and energy, cyanidin-3-O-glucoside might have a biological function as an inhibitor of AGEs-RAGE interactions, which was more likely through the establishment of AGEs-cyanidin-3-O-glucoside-RAGE. Conclusion: This study suggests that cyanidin-3-O-glucoside in red rice can be a potential AGEs-RAGE inhibitor, leading to the regulation of the pro-inflammatory and oxidative damage in the cellular pathway. Keywords: argypirimidine, cyanidin 3-O-glucoside, imidazole, pentosidine, pyrralline

1. INTRODUCTION

Advanced glycation end products (AGEs) are the biomarkers of hyperglycemia and pro-inflammatory, which result from the non-enzymatic glycation of protein and sugar (1). The endogenous formation of AGEs involves arginine, lysine, and sulfur-containing amino acids, which are prone to glycoxidation; lipids; as well as glucose, ribose, mannose, and reactive triose intermediates that are sensitive to oxidation and degradation (2,3). AGEs can also be produced from exogenous sources, such as high fat and high sugar diets processed in high temperature (4). Different AGEs compounds can be classified as fluorescent cross-linking AGEs, such as pentosidine, glyoxal-lysine dimer, methylglyoxal-lysine dimer; and non-fluorescent non-cross-linking AGEs such as pyrralline, argypirimidine and Pyrralline, could bound to RAGE at the same residue as those two AGEs did with the binding energy of -411.8 kcal/mol and -1305 kcal/mol, respectively. Based on the binding site location and energy, cyanidin-3-O-glucoside might have a biological function as an inhibitor of AGEs-RAGE interactions, which was more likely through the establishment of AGEs-cyanidin-3-O-glucoside-RAGE.

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2. METHODS

Protein and Ligand Preparation

Proteins and ligands were obtained from the database of RCSB Protein Data Bank (PDB) (http://www.rcsb.org/structure/3O3U) and PubChem (http://pubchem.ncbi.nlm.nih.gov/). The RAGE (PDB ID: 3O3U) was used in this study. The ligands and water molecules incorporated in its 3D structures were removed using Discovery Studio Ver. 4.1 software. The ligands were cyanidin-3-O-glucoside (CID 12303203) and several AGEs, namely, argypirimidine (CID 17750123), imidazole (CID 795), pyrraline (CID 122228), and pentosidine (CID 119593). The energy minimization was performed for each ligand using PyRx Ver. 0.8 software. The SDF file format of each ligand was converted to PDB format using Open Babel.

Ligand-Protein Docking and Visualization

Each of AGE (argypirimidine, imidazole, pyrraline, and pentosidine) was assessed for the ability to bind RAGE and or cyanidin-3-O-glucoside. We would like to compare the interactions that occurred between the AGE-RAGE complex with and without the presence of cyanidin-3-O-glucoside. The possibility of cyanidin-3-O-glucoside to bind AGE, leading to the prevention of AGEs interaction with RAGE, was also investigated. Therefore, the docking of AGE-RAGE, AGE-cyanidin-3-O-glucoside, AGE-RAGE-cyanidin-3-O-glucoside, and AGE-cyanidin-3-O-glucoside-RAGE were established using Hex 8.0 software with Shape + Electro + DARS mode. Amino acid residues, types and binding energies of receptor-ligand interactions were also identified using this software. The visualization of the molecular interactions was performed using Discovery Studio Ver. 4.1 software.
3. RESULTS

The Possible Interactions formed in the Binding of AGEs-RAGE

Interactions between AGEs (argypirimidine, pentosidine, pyrralline, and imidazole) and RAGE had maintained by hydrogen and hydrophobic bonds (Table 1A). Residues involved in argypirimidine-RAGE interactions were LYS42, LYS46, GLY1213, LEU1214, and ARG1218 (Figure 1A). Pentosidine could bind to ALA52, ASN1025, and PRO1215 of RAGE (Figure 1B). In the pyrralline-RAGE complex, the amino acid residues involved were TRP62, ALA63, TRP230, LEU262, and LEU299 (Figure 1C). There were four amino acid residues of RAGE that capable of interacting with imidazole, namely ALA63, ASP65, MET330, and TRP340 (Figure 1D). The binding energy of the AGEs-RAGE interaction from the lowest to the highest was the complex of pyrralline-RAGE, pentosidine-RAGE, argypirimidine-RAGE, and imidazole-RAGE with the energy of -591.1 kcal/mol, -350.4 kcal/mol, -247 kcal/mol, and -100.4 kcal/mol, respectively.

The Interaction of AGEs-RAGE Complex with Cyanidin-3-O-glucoside

The binding of AGEs-RAGE complex with Cyanidin-3-O-glucoside was identified by the bonds formed, the type of bond, and the binding energy (Table 1B). The AGEs-RAGE-cyanidin-3-O-glucoside complex analysis showed that argypirimidine, pentosidine, pyrralline, and imidazole could still bind to RAGE at the same position as in the complex of AGEs-RAGE (Figure 1A-C). Interestingly, cyanidin-3-O-glucoside could bind to the ALA63 of RAGE in the imidazole-RAGE-cyanidin-3-O-glucoside complex. Imidazole was reported to interact in this residue as well. This result indicated that cyanidin-3-O-glucoside was able to compete with imidazole in binding the RAGE (Figure 2D). The docking of the imidazole-RAGE complex with cyanidin-3-O-glucoside had the energy binding of -299 kcal/mol, which was lower than the energy needed in the interaction of imidazole and RAGE. Therefore, cyanidin-3-O-glucoside is easy to interact with the Imidazole-RAGE complex.

The Interaction of AGEs with Cyanidin-3-O-glucoside

Chemical bonds and binding energy of the interaction of AGEs-cyanidin-3-O-glucoside are presented in Table 2A. The hydrogen and hydrophobic bonds stabilized the binding of cyanidin-3-O-glucoside and each of the four AGEs(Figure 3A1-D1). The binding energy needed in those interactions from the lowest to the highest was -164 kcal/mol (argypirimidine-cyanidin-3-O-glucoside complex), -163.2 kcal/mol (pentosidine-cyanidin-3-O-glucoside complex), -150.3 kcal/mol (pyrralline-cyanidin-3-O-glucoside complex), and -95.7 kcal/mol (imidazole-cyanidin-3-O-glucoside complex). Of four AGEs, the binding of argypirimidine and cyanidin-3-O-glucoside was the strongest due to the highest amount of chemical bonds, and the two hydrogen bonds stabilized them.

The Binding Analysis of AGEs-Cyanidin-3-O-glucoside

The possible interactions may be responsible for the biological activity of AGEs in the prevention of age-related diseases. For instance, the binding of cyanidin-3-O-glucoside with argypirimidine-RAGE complex showed that the hydrogen bond and hydrophobic bond were formed. Similarly, the binding of cyanidin-3-O-glucoside with pentosidine-RAGE complex showed that the hydrogen bond and hydrophobic bond were formed. Therefore, cyanidin-3-O-glucoside is easy to interact with the Imidazole-RAGE complex.
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The molecular docking result for the AGEs-cyanidin-3-O-glucoside complex with RAGE is presented in Table 2B. Our study suggested that cyanidin-3-O-glucoside might compete with the two AGEs, argypirimidine and pyrraline. Cyanidin-3-O-glucoside was able to bind RAGE at the same residue as argypirimidine and pyrraline did. In argypirimidine-cyanidin-3-O-glucoside-RAGE complex, hydrophobic bonds maintained the interaction of argypirimidine with ALA52 and TYR341 of RAGE. Cyanidin-3-O-glucoside also established a hydrophobic interaction with the ALA52 and a hydrogen bond with TYR341 (Figure 3A2). In pyrraline-cyanidin-3-O-glucoside-RAGE complex, the TRP230 of RAGE was involved in the interaction with pyrraline through hydrogen bond. Cyanidin-3-O-glucoside had hydrophobic interactions with this residue as well (Figure 3C2). Cyanidin-3-O-glucose can bind to RAGE and compete with imidazole in the imidazole-RAGE-cyanidin-3-O-glucose complex. However, cyanidin-3-O-glucose, which was first bound to Imidazole, could not interact with RAGE at all (Figure 3D2).

Each of four AGEs that connected with cyanidin-3-O-glucoside had different binding sites in RAGE compared with the interactions that occurred in the AGEs-RAGE complex (Figure 3A2-D2). This study implied that the presence of cyanidin-3-O-glucoside could affect the AGEs-RAGE interaction. The complex of pyrraline-cyanidin-3-O-glucoside-RAGE, pentosidine-cyanidin-3-O-glucoside-RAGE, argypirimidine-cyanidin-3-O-glucoside-RAGE, and imidazole-cyanidin-3-O-glucoside-RAGE showed the binding energy of -1305 kcal/mol, -462.2 kcal/mol, -411.8 kcal/mol, and 108.4 kcal/mol, respectively (Table 2B). The binding of the pyrraline-cyanidin-3-O-glucoside-RAGE complex had the strongest bond because it required the smallest binding energy. In addition, its interactions were stabilized by six hydrogen bonds and more chemical bonds than other complexes. Therefore, cyanidin-3-O-glucoside could be a potential inhibitor of AGEs-RAGE interaction through its binding to RAGE at the same amino acid residue with AGEs showed in the complex of AGE-Cyanidin-3-O-glucoside-RAGE.

4. DISCUSSION

RAGE is a transmembrane protein, which consists of three different extracellular domains (a V-type domain (residue 23-116), CI domain (residue 124-221), and C2 domain (residue 227-317)), a transmembrane helix (residue 345-363), and a cytoplasmic tail (564-404) (18) including vascular diseases, cancer, neurodegeneration and diabetes. Its oligomerization is believed to be important in signal transduction, but RAGE oligomeric structures and stoichiometries remain unclear. Different oligomerization modes have been proposed in studies involving different truncated versions of the extracellular parts of RAGE. Here, we provide basic characterization of the oligomerization patterns of full-length RAGE (including

![Figure 2. Visualization of AGEs-RAGE-Cyanidin-3-O-glucoside molecular docking. AGEs are shown in dark blue color. RAGE is shown in light blue color. Cyanidin-3-O-glucoside is in red color.](image-url)
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the transmembrane (TM). This study showed that argypirimidine, pentosidine, pyrralline, and imidazole could interact with RAGE in the V and C domain. Previous study also reported that the four AGEs bound to the C domain of RAGE (6) cardiovascular disease, stroke, neuropathy, and nephropathy. Different studies have been done to employ AGEs as drug targets for the diseases therapy. In previous study, we have found bioactive peptide from Ethawah goat milk for anti-diabetic that may work through inhibition of AGE receptor function. However, the mechanism of bioactive peptides inhibits AGE-AGE receptor (RAGE). The V domain is a ligand-binding domain that has a role in the extracellular and intracellular signal transduction. The C domain plays an important as a mediator that supports the function of V domain (19,20)amyloid fibrils, amphoterins and S100/calgranulins. The overlapping distribution of these ligands and cells overexpressing RAGE results in sustained receptor expression which is magnified via the apparent capacity of ligands to upregulate the receptor. We hypothesize that RAGE-ligand interaction is a propagation factor in a range of chronic disorders, based on the enhanced accumulation of the ligands in diseased tissues. For example, increased levels of AGEs in diabetes and renal insufficiency, amyloid fibrils in Alzheimer’s disease brain, amphoterin in tumors and S100/calgranulins at sites of inflammation have been identified. The engagement of RAGE by its ligands can be considered the ‘first hit’ in a two-stage model, in which the second phase of cellular perturbation is mediated by superimposed accumulation of modified lipoproteins (in atherosclerosis).

RAGE is activated through the binding with AGEs that cause the stimulation of signal transduction and the activation of NF-κB. NF-κB activates transcription genes to secrete pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6. It also causes the increase of vascular cell adhesiveness and oxidative stress that can cause vascular complications of diabetes (9). The binding of AGEs (argypirimidine, pentosidine, pyrralline, and imidazole) with RAGE can modulate the complex cellular signaling cascade leading to inflammation and oxidative stress which trigger the development of the pathogenesis of diabetes, cardiovascular, atherosclerosis, stroke, and cancer (21–23)the products of nonenzymatic glycoxidation of proteins and lipids. The finding that AGEs stimulate signal transduction cascades through the multiligand receptor RAGE unveiled novel insights into diabetes and its complications. Inextricably woven into AGE-RAGE interactions in diabetes is the engagement of the innate and adaptive immune responses. Although glucose may be the triggering stimulus to draw RAGE into diabetes pathology, consequent cellular stress results in release of proinflammatory RAGE ligands S100/calgranulins and HMGB1. We predict that once RAGE is engaged in the diabetic tissue, a vicious cycle of ligand-RAGE perturbation ensues, leading to chronic tissue injury and suppression of repair mechanisms. Targeting RAGE may be a beneficial strategy in diabetes, its complications, and untoward inflammatory responses. Receptor for AGE (RAGE). The interaction of the four AGEs with RAGE is stabilized by hydrogen and hydrophobic bonds. When the hydrogen atoms of a molecule bind to other molecules that are more electronegative, the hydrogen bonds are created (24). These bonds play a role to stabilize the
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#### A. Interaction of Advanced Glycation End Products (AGEs)-receptor (RAGE)

| Interaction          | Point interaction | Donor atom | Acceptor atom | Type               | Chemistry bond                        | Energy binding (kcal/mol) |
|----------------------|-------------------|------------|---------------|--------------------|--------------------------------------|---------------------------|
| **Argpyrimidine-RAGE** |                   |            |               |                    |                                      |                           |
| Argypirimidine-RAGE  | N:ARG1218:HH11    | LIG1:OXT  | LIG1:H        | Conventional Hydrogen Bond | Hydrogen Bond                        | -247                      |
|                      | N:GLY1213:O       | LIG1:H    | N:GLY1213:O   | Carbon Hydrogen Bond  | Hydrogen Bond                        |                           |
|                      | N:GLY1213:O       | LIG1:H    | N:GLY1213:O   | Carbon Hydrogen Bond  | Hydrogen Bond                        |                           |
|                      | N:LYS1542         | LIG1:C    | N:LYS1542     | Alkyl              | Hydrophobic                          |                           |
|                      | N:LYS1546         | LIG1:C    | N:LYS1546     | Alkyl              | Hydrophobic                          |                           |
|                      | N:LYS1546         | LIG1      | N:LYS1546     | Pi-Alkyl           | Hydrophobic                          |                           |
| **Pentosidine-RAGE** |                   |            |               |                    |                                      |                           |
| Pentosidine-RAGE     | N:ASN1025:HD21    | LIG1:O    | LIG1:H        | Conventional Hydrogen Bond | Hydrogen Bond                        | -350.4                    |
|                      | N:ASN1025:HD21    | LIG1      | N:ALAS2      | Pi-Alkyl           | Hydrophobic                          |                           |
|                      | N:LY262:HN        | LIG1:O    | LIG1:H        | Conventional Hydrogen Bond | Hydrogen Bond                        | -591.1                    |
|                      | N:LY262:HN        | LIG1      | N:ALAS2      | Pi-Alkyl           | Hydrophobic                          |                           |
|                      | N:LY262:HN        | LIG1      | N:ALAS2      | Pi-Alkyl           | Hydrophobic                          |                           |
|                      | N:LY262:HN        | LIG1      | N:ALAS2      | Pi-Alkyl           | Hydrophobic                          |                           |
| **Imidazole-RAGE**   |                   |            |               |                    |                                      |                           |
| Imidazole-RAGE       | N:LY1025:HD21     | LIG1:O    | LIG1:H        | Conventional Hydrogen Bond | Hydrogen Bond                        | -100.4                    |
|                      | N:LY1025:HD21     | LIG1      | N:ALAS2      | Pi-Sulfur          | Other                                |                           |
|                      | N:LY1025:HD21     | LIG1      | N:ALAS2      | Pi-Sulfur          | Other                                |                           |
|                      | N:LY1025:HD21     | LIG1      | N:ALAS2      | Pi-Sulfur          | Other                                |                           |
|                      | N:LY1025:HD21     | LIG1      | N:ALAS2      | Pi-Sulfur          | Other                                |                           |

#### B. Interaction of AGEs-RAGE Complex with Cyanidin-3-O-glucoside

| Interaction          | Point interaction | Donor atom | Acceptor atom | Type               | Chemistry bond                        | Energy binding (kcal/mol) |
|----------------------|-------------------|------------|---------------|--------------------|--------------------------------------|---------------------------|
| **Argpyrimidine-RAGE** |                   |            |               |                    |                                      |                           |
| Argypirimidine-RAGE  | N:ARG1218:HH11    | LIG1:OXT  | LIG1:H        | Conventional Hydrogen Bond | Hydrogen Bond                        | -301.4                    |
|                      | N:LY1213:O        | LIG1:H    | N:LY1213:O    | Carbon Hydrogen Bond  | Hydrogen Bond                        |                           |
|                      | N:GLY1213:O       | LIG1:H    | N:GLY1213:O   | Carbon Hydrogen Bond  | Hydrogen Bond                        |                           |
|                      | N:LYS1542         | LIG1:C    | N:LYS1542     | Alkyl              | Hydrophobic                          |                           |
|                      | N:LYS1546         | LIG1:C    | N:LYS1546     | Alkyl              | Hydrophobic                          |                           |
|                      | N:LYS1546         | LIG1      | N:LYS1546     | Pi-Alkyl           | Hydrophobic                          |                           |
| **Pentosidine-RAGE** |                   |            |               |                    |                                      |                           |
| Pentosidine-RAGE     | N:ASN1025:HD21    | LIG1:O    | LIG1:H        | Conventional Hydrogen Bond | Hydrogen Bond                        | -307.2                    |
|                      | N:ASN1025:HD21    | LIG1      | N:PR0154      | Pi-Alkyl           | Hydrophobic                          |                           |
|                      | N:ASN1025:HD21    | LIG1      | N:PR0154      | Pi-Alkyl           | Hydrophobic                          |                           |
|                      | N:ASN1025:HD21    | LIG1      | N:PR0154      | Pi-Alkyl           | Hydrophobic                          |                           |
|                      | N:ASN1025:HD21    | LIG1      | N:PR0154      | Pi-Alkyl           | Hydrophobic                          |                           |

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The binding affinity of the imidazole-RAGE-cyanidine-3-O-glucoside complex was also smaller than that of the imidazole-RAGE complex, which was -299 kcal/mol. Previous study, reported that that the interaction and position between ligand-receptors after binding to the bioactive compound affected the binding energy (6)cardiovascular disease, stroke, neuropathy, and nephropathy. Different studies have been done to employ AGEs as drug targets for the diseases therapy. In previous study, we have found bioactive peptide from Ethanaw goat milk for anti-diabetic that may work through inhibition of AGE receptor function. However, the mechanism of bioactive peptides inhibits AGE- AGE receptor (RAGE). The binding of cyanidin-3-O-glucoside to AGEs-RAGE complex also influenced the binding energy in the AGEs-RAGE-cyanidin-3-O-glucoside complex in this study. However, cyanidin-3-O-glucose complexes with imidazole could not bind to RAGE. It might be inferred that the cyanidin-3-O-glucose could inhibit the imidazole-RAGE interaction more effectively by the establishment of imidazole-RAGE-cyanidin-3-O-glucoside complex than in the imidazole-cyanidin-3-O-glucoside-RAGE complex.

This study reported that argyrrimidine and cyanidin-3-O-glucoside could establish the interactions with ALA52 and TYR341 of RAGE showed in the argyrrimidine complex was also than in the imidazole-cyanidin-3-O-glucoside-RAGE complex. Cyanidin-3-O-glucoside was also capable of binding the TRP230 of RAGE, which was the residue bound to pyrralline, in the pyrralline-cyanidin-3-O-glucoside-RAGE complex. Our results showed that cyanidin-3-O-glucoside might have a biological function as a competitive inhibitor of both AGEs in binding to RAGE. The binding affinity indicates the less energy needed to form chemical bonds with the other molecules. The smaller the binding affinity indicates the less energy needed between molecules to interact. Therefore, they can interact with each other easily (27).

A compound is a competitive inhibitor when it can compete with the substrate to bind to the active site of the enzyme. The interaction between the inhibitor and the enzyme will prevent the bonding between the enzyme and the substrate (28). One of the factors that influence a competitive inhibitor in inhibiting the interaction between two proteins efficiently is the binding affinity of inhibitors (29). This research found that cyanidin-3-O-glucoside can compete with imidazole through binding the ALA63 of RAGE in the complex of imidazole-RAGE with Cyanidin-3-O-glucoside. Therefore, imidazole-RAGE interaction might be inhibited.

### Table 1: Interaction of Advanced Glycation End Products (AGEs)-receptor (RAGE) and Cyanidin-3-O-glucoside

| N:LEU262:HN - N:LIG1:H | N:LEU262:HN - N:LIG1:H | Conventional Hydrogen Bond | Hydrogen Bond |
|------------------------|------------------------|-----------------------------|--------------|
| N:LIG1:H - N:LEU299:O  | N:LIG1:H - N:LEU299:O  | Conventional Hydrogen Bond | Hydrogen Bond |
| N:LIG1:H - N:TRP230    | N:LIG1:H - N:TRP230    | Pi-Sigma Hydrophobic        |              |
| N:LIG1 - N:TRP62       | N:LIG1 - N:TRP62       | Pi-T-shaped Hydrophobic     |              |
| N:LIG1 - N:TRP230      | N:LIG1 - N:TRP230      | Pi-T-shaped Hydrophobic     |              |
| N:LIG1 - N:ALA63       | N:LIG1 - N:ALA63       | Pi-Alkyl Hydrophobic        |              |

**Pyrralline-RAGE-Cyanidin-3-O-glucoside**

- **N:LIG1:H - N:ASP65:OD1**: Carbon Hydrogen Bond, Hydrogen Bond
- **N:LIG1:H - N:ASP65:OD2**: Carbon Hydrogen Bond, Hydrogen Bond
- **N:MET336:CE - N:LIG1**: Pi-Sigma Hydrophobic
- **N:MET336:SE - N:LIG1**: Pi-Sigma Hydrophobic
- **N:TRP340 - N:LIG1**: Pi-Stacked Hydrophobic
- **N:LIG1 - N:TRP340**: Pi-Stacked Hydrophobic
- **N:LIG1 - N:TRP340**: Pi-Stacked Hydrophobic
- **N:LIG1 - N:TRP340**: Pi-Stacked Hydrophobic
- **N:LIG1 - N:ALA63**: Pi-Alkyl Hydrophobic

**Imidazole-RAGE-Cyanidin-3-O-glucoside**

- **N:LIG1:O - N:GLU153:OE1**: Pi-Anion Electrostatic
- **N:LIG1:O - N:GLU153:OE1**: Pi-Anion Electrostatic
- **N:LIG1 - N:TRP62**: Pi-Sulfur Hydrophobic
- **N:LIG1 - N:TRP62**: Pi-Sulfur Hydrophobic
- **N:LIG1 - N:TRP62**: Pi-Sulfur Hydrophobic
- **N:LIG1 - N:TRP62**: Pi-Sulfur Hydrophobic
- **N:LIG1 - N:TRP62**: Pi-Sulfur Hydrophobic
- **N:LIG1 - N:TRP62**: Pi-Sulfur Hydrophobic

**RAGE-Cyanidin-3-O-glucoside**

- **N:LEU148:HN - N:LIG1:H**: Pi-Alkyl Hydrophobic
- **N:LEU148:HN - N:LIG1:H**: Pi-Alkyl Hydrophobic
- **N:LEU148:HN - N:LIG1:H**: Pi-Alkyl Hydrophobic
- **N:LEU148:HN - N:LIG1:H**: Pi-Alkyl Hydrophobic
- **N:LEU148:HN - N:LIG1:H**: Pi-Alkyl Hydrophobic
- **N:LEU148:HN - N:LIG1:H**: Pi-Alkyl Hydrophobic
- **N:LEU148:HN - N:LIG1:H**: Pi-Alkyl Hydrophobic

**RAGE-Imidazole-Cyanidin-3-O-glucoside**

- **N:LEU148:HN - N:LIG1:H**: Pi-Alkyl Hydrophobic
- **N:LEU148:HN - N:LIG1:H**: Pi-Alkyl Hydrophobic
- **N:LEU148:HN - N:LIG1:H**: Pi-Alkyl Hydrophobic
- **N:LEU148:HN - N:LIG1:H**: Pi-Alkyl Hydrophobic
- **N:LEU148:HN - N:LIG1:H**: Pi-Alkyl Hydrophobic
- **N:LEU148:HN - N:LIG1:H**: Pi-Alkyl Hydrophobic
- **N:LEU148:HN - N:LIG1:H**: Pi-Alkyl Hydrophobic

**Pyrralline-RAGE-Cyanidin-3-O-glucoside**

- **N:LEU148:HN - N:LIG1:H**: Pi-Alkyl Hydrophobic
- **N:LEU148:HN - N:LIG1:H**: Pi-Alkyl Hydrophobic
- **N:LEU148:HN - N:LIG1:H**: Pi-Alkyl Hydrophobic
- **N:LEU148:HN - N:LIG1:H**: Pi-Alkyl Hydrophobic
- **N:LEU148:HN - N:LIG1:H**: Pi-Alkyl Hydrophobic
- **N:LEU148:HN - N:LIG1:H**: Pi-Alkyl Hydrophobic
- **N:LEU148:HN - N:LIG1:H**: Pi-Alkyl Hydrophobic

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An in Silico Approach Reveals the Potential Function of Cyanidin-3-o-glucoside of Red Rice

The energy of the argypirimidine-cyanidin-3-O-glucoside-RAGE complex was -411.8 kcal/mol, lower than the argypirimidine-RAGE complex. The pyrralline-cyanidin-3-O-glucoside-RAGE complex also had the binding energy of -1305 kcal/mol. It was lower than the binding energy of the pyrralline-RAGE complex. Moreover, the binding sites of the four AGEs and RAGE were changed in the presence of cyanidin-3-O-glucoside. This result implied that cyanidin-3-O-glucoside might interfere with the AGEs so that they could not bind to certain sites of RAGE. Ligand binding, hydrogen bonding, and hydrophobic interactions were important factors contributing to the binding of AGEs and cyanidin-3-O-glucoside.

Table 2. Interaction of AGEs-Cyanidin-3-O-glucoside and receptor AGEs

| Interaction | Point interaction | Donor atom | Acceptor atom | Type | Chemistry bond | Energy binding (kcal/mol) |
|-------------|-------------------|------------|---------------|------|----------------|--------------------------|
| A. Interaction of AGEs and Cyanidin-3-O-glucoside | LIG1:H - LIG1:N | LIG1:H | LIG1:N | Conventional Hydrogen Bond | Hydrogen Bond | -164 |
|             | LIG1:H - LIG1:O  | LIG1:H | LIG1:O | Carbon Hydrogen Bond | Hydrogen Bond | -163.2 |
|             | LIG1:N - LIG1    | LIG1:N | LIG1   | Pi-Lone Pair | Other | -150.3 |
|             | LIG1 - LIG1      | LIG1   | LIG1   | Pi-Pi Stacked | Hydrophobic | -95.7 |
| B. Interaction of AGEs-Cyanidin-3-O-glucoside Complex with RAGE | N:GLN1024:HA - LIG1:O | N:GLN1024:HA | LIG1:O | Carbon Hydrogen Bond | Hydrogen Bond | -411.8 |
|             | LIG1:C - N:LEU1214:HA | LIG1:C | N:LEU1214:HA | Alkyl | Hydrophobic | -462.2 |
|             | N:TYR341 - LIG1:C | N:TYR341 | LIG1:C | Pi-Alkyl | Hydrophobic | -1304.9 |
|             | LIG1 - N:ALA52   | LIG1   | N:ALA52 | Pi-Aromatic | Hydrophobic | -108.4 |

Table 2. Interaction of AGEs-Cyanidin-3-O-glucoside and receptor AGEs

energy of the argypirimidine-cyanidin-3-O-glucoside-RAGE complex was -411.8 kcal/mol, lower than the argypirimidine-RAGE complex. The pyrralline-cyanidin-3-O-glucoside-RAGE complex also had the binding energy of -1505 kcal/mol. It was lower than the binding energy of the pyrralline-RAGE complex. Moreover, the binding sites of the four AGEs and RAGE were changed in the presence of cyanidin-3-O-glucoside. This result implied that cyanidin-3-O-glucoside might interfere with the AGEs so that they could not bind to certain sites of RAGE. Ligand binding, hydrogen bonding, and hydrophobic interactions were important factors contributing to the binding of AGEs and cyanidin-3-O-glucoside.
drophobic effects may change the overall fold or protein conformation, leading to a nonfunctional protein (30). In this case, the AGEs-RAGE signaling might be impeded by cyanidin-3-O-glucoside.

Cyanidin-3-O-glucoside is an anthocyanin that can be found in pigmented rice, including red rice. It has two hydroxyls on the B ring and hydrogen donor or acceptor in its structure so that it has the potential to have strong antioxidant activity (31–34). Different bran of Thai rice cultivars which were divided into 3 groups: white color (Hom mali 105, Supan, Saochai, Hom chaiya and Hom jun. The heating process in food contributes to the accumulation of AGES. Frying and roasting can increase AGES content higher than steaming and boiling. Consuming foods with proper processing, such as whole grains that are rich in vitamins and antioxidants, can inhibit the formation of AGES (35). This study indicated that cyanidin-3-O-glucoside might act as a competitive inhibitor of AGES (argyprimidine, pentosidine, and pyrralline), leading to the potential ability in inhibiting the interaction of AGES and RAGE through the establishment of AGES-Cyanidin-3-O-glucoside-RAGE complex.

5. CONCLUSION

Cyanidin-3-O-glucoside in red rice may have a potential compound as an inhibitor of AGES-RAGE signaling. This was shown from the stable complex formed of AGES-cyanidin-3-O-glucoside-RAGE.

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