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The *in silico* study of phytoestrogenic activity of soy in substitution of estrogen function

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ABSTRACT

Backgroud: Isoflavone compounds in soy are known as phytoestrogen compounds. These compounds are expected to have important roles in replacing estrogen role in menopausal women.
Aim: The purposes of this study were to investigate the estrogen receptor (ER) modulation by soy isoflavone compounds and how the estrogen compound takes a role in gene synthesis related to proliferation and apoptosis.
Methods: The analysis was performed *in silico* manner in which docking as the most important method was carried out using Hex 8.0 software and HADDOCK webserver. Interaction analysis was then done to observe the interactions between soy isoflavone compound and several related proteins using the softwares of Discovery Studio, LigPlus, and NUCPLOT.
Results: The results of this study indicated that soy isoflavone compounds have the ability to bind to ERs.
Conclusions: It can be concluded that soy isoflavone compounds can serve as phytoestrogen that can activate ER.

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KEYWORDS

Daidzein; estrogen; estrogen receptor; genistein; glycitein; *in silico*

Introduction

Estrogen regulates the differentiation and maintains reproductive tissue, muscle, and other tissues by activating its receptor [1]. The structural study indicates that estrogen and antagonist estrogen compound can induce conformational change in different estrogen receptor alpha (ERα), in which this conformational change determines the recruitment of coactivator or corepressor, leading to diverse biological effects [2]. The most conserved domain of ER is DNA-binding domain which is involved in recognition of DNA and binding to DNA, whereas the ligand binding occurs at ligand-binding domain in COOH-terminal region [3]. ERα and ERβ have high sequence homology level. However, their NH2-terminal domains have similar affinity level to estrogen, and bind to the same DNA response element [4].

Some plants produce compound that has estrogenic activity, so they are called as phytoestrogen compounds. These compounds have similar structure to estrogen. They also have phenolic ring, which is needed for the binding process with ER [5]. Phytoestrogen is contained in food in the forms of aglycone and glucoside. The currently recognized main phytoestrogens are soy isoflavone compounds, such as genistein, daidzein, and glycitein, and the glycoside (genistin, daidzin, and glycitin). Many studies have reported that these phytoestrogen compounds have larger affinity level on ERβ compared with ERα [5]. The genistein has 1,000 times more potential in inducing transcriptional activity in ERβ [6]. Thus, genistein has preference in cells which mainly express ERβ compared with cells that mainly express ERα.

Many studies have reported that the estrogenic effect of soy isoflavones is relatively small

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(1/1,000–1/100,000 compared with estradiol activity), these compounds supposedly have agonistic effect on estrogen. When the estrogen is in abundant amount, the soy isoflavones are assumed to be able to act as anti-estrogen by competing to bind to ER in cells. However, when the estrogen is in very small quantity (menopause), soy isoflavones supposedly have estrogenic effect by replacing the estrogen hormone functions and some of them acts to reduce the osteoporosis symptom, reducing the risks of cardiovascular disease and osteoporosis [7]. This study aimed to focus on the ER modulation by soy isoflavone compounds.

Materials and Methods

Nucleotide sequence and protein structure retrieval

The structures of the components of active compound in soy were obtained from PubChem, an open chemistry database. Three active compounds were analyzed, including daidzein (CID 5281708), genistein (CID 5280961), and glycitein (CID 5317750). Protein sequences of ERα (GI: 262117988) and ERβ (GI: 6978817) were obtained from the sequences database of National Center for Biotechnology Information, the United States National Library of Medicine, and National Institute of Health (http://www.ncbi.nlm.nih.gov).

3D-structural modeling of DNA, protein, and bioactive component

3D-structural modeling of ERα and ERβ was predicted using SWISS-MODEL webserver [8,9] by homology modeling method. 3D structure of protein was then validated using Ramachandran plot analysis. 3D-structural modeling of Hsp70 and Bcl-xL gene promoters was carried out using 3D-DART webserver. Conversion of *.sdf file to *.pdb file of the soy active component was performed using OpenBabel software [10].

Computational docking

Docking simulation was done using HEX 8.0 software [11]. Docking protocol consists of three visualization stages: rigid-body energy minimization, semi-flexible repair, and finishing refinement in explicit solvent. After the execution of each stage, the docking confirmation was then scored and sorted based on scoring function to facilitate the selection of best conformation that will be used at the next stage.

Inter-protein interaction analysis

The docking analysis results are then visualized using Discovery Studio 4.1, LigPlot+ [12], and LigandScout 3.1 softwares [13], while the visualization and interaction analysis between protein and DNA were executed using NUCPLOT software. Interaction analysis was done to observe the formed bonds, such as hydrogen, hydrophobic, and van der Waals bonds. Pharmacophore analysis was also conducted to detect the residues that were directly involved in interaction process, and energy minimization analysis was done to repair the molecular structure and shape at the time of interaction.

Results

Docking analysis aimed to find out the possible interaction between soy active compounds including daidzein, genistein, and glycitein to bind to ER (ERα and ERβ). The analysis of possible interaction was done because one of the compounds in soy is phytoestrogen compound. Of the three soy active compounds analyzed here, glycitein is the most easily interacted compound with ER. Glycitein required an energy of −254.81 kJ/mol to interact

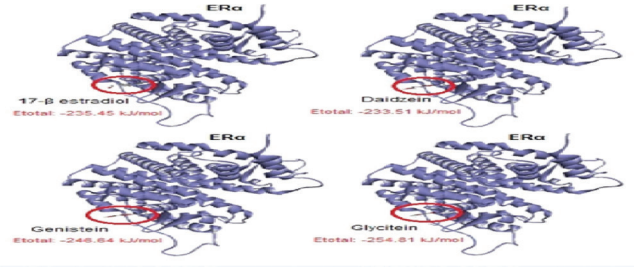

Figure 1. The interaction between estrogen and soy isoflavone compound with ERα. Glycitein required an energy of −254.81 kJ to interact with ERα, in which this energy was smaller than the energy required by estrogen (17β estradiol) to bind (−235.45 kJ/mol).

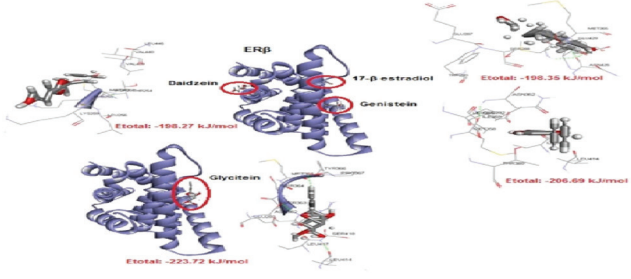

Figure 2. The interaction between estrogen and soy isoflavone compound with ERβ. Glycitein required an energy of −223.72 kJ/mol to interact with ERβ, in which this energy was smaller than the energy required by estrogen (17β estradiol) to bind (−198.35 kJ/mol).

Table 1. Possible interactions between soy active compounds and ERα.

Molecules	Point interaction	Category	Distance (Å)	Binding energy (kJ/mol)
ERα—17β estradiol	17β estradiol: O—ILE391: O	Hydrogen bond	2,406	−235.45
ERα—Daidzein	Daidzein: O—ILE391: O	Hydrogen bond	2,823	−233.51
ERα—Genistein	Genistein: O—ILE391: O	Hydrogen bond	3,134	−246.64
ERα—Glycitein	Glycitein: O—ILE391: O	Hydrogen bond	1,979	−254.81

Table 2. Possible interactions between soy active compounds and ERβ.

Molecules	Point interaction	Category	Distance (Å)	Binding energy (kJ/mol)
ERβ—estradiol 17β	17β estradiol: O—ASN425: O	Hydrogen bond	3,121	−198.35
ERβ—Daidzein	Daidzein: O—Met251: O	Hydrophobic bond	3,167	−198.27
	Daidzein: O—Thr254: O	Hydrophobic bond	2,221	
ERβ—Genistein	Genistein: O—Met358	Hydrophobic bond	3,347	−206.69
	Genistein: O—Leu385	Hydrophobic bond	3,293	
ERβ—Glycitein	Glycitein: O—TYR366: O	Hydrogen bond	2,747	−223.72

with ERα, in which this energy was smaller than the energy required by estrogen (17β estradiol) to bind (−235.45 kJ/mol) (Table 1 and Fig. 1). Glycitein also required less energy (−223.72 kJ/mol) to bind to ERβ compared with estrogen or other active compounds (Table 2 and Fig. 2).

Discussion

The study focused on three main ingredients (daidzein, genistein, and glycitein) based on bioavailability and estrogenic activity of these three materials were higher than the isoflavone-glycoside conjugate form (daidzin, genistin, and glycitin) [14]. Isoflavone concentrations in soy vary depending on a variety of environmental, genetic, harvesting, and processing conditions, but the ratio deidzein, genistein, and glycitein is 1:1:0.1 [15].

Soy glycitein compound is expected to have the highest affinity to ERα and ERβ compared with other soy isoflavone compound. Besides the glycitein, genistein also has fairly high affinity to ERβ. This bond supposedly can replace the estrogen role when a woman experiences estrogen deficiency. Phytoestrogen compound in soy is bound by ER. The bond between phytoestrogen compound and ER activates the intracellular signaling pathway that starts from the activation of phospholipase-C enzyme. This enzyme changes the phosphatidylinositol bisphosphate (PI-2p) to phosphatidylinositol triphosphate (PI-3p). The bond between PI-3p and its receptor located on the surface of endoplasmic reticulum results in the opening of calcium gate; thus, the intracellular calcium ion increases. The calcium ion binds to calcineurin in cytosol. The existence of this calcineurin complex will inhibit the activity of kappa beta (Iκ-β) inhibitor, so the nuclear factor kappa beta (NF-κβ) then translocates to cell nucleus and trigger the transcription of target genes [16,17].

Classic action of estrogen and phytoestrogen isoflavones is mediated through the transcription activation of the genes which are responsive to estrogen, including the intracellular ER [16]. The hormone-receptor complex binds to the estrogen responsive element in promoter region of target gene, thereby inducing the transcription process of such genes. However, the activation of target gene by estrogen may also be mediated by other transcription factor protein, including activating protein 1 and NF-κβ [16].

Based on the results of this study, it can be concluded that soy isoflavone compounds can serve as phytoestrogen that can activate ER.

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