J Ginseng Res 44 (2020) 690–696

Contents lists available at ScienceDirect

Journal of Ginseng Research

journal homepage: http://www.ginsengres.org

Research Article

Computational and experimental characterization of estrogenic activities of 20(*S*, *R*)-protopanaxadiol and 20(*S*, *R*)-protopanaxatriol

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ARTICLE INFO

Article history:
Received 31 December 2017
Received in Revised form
24 March 2018
Accepted 8 May 2018
Available online 1 June 2018

Keywords:
Estrogenicity
Fluorescence polarization
Ginsenosides
Molecular modeling
Reporter gene assay

ABSTRACT

Background: As the main metabolites of ginsenosides, 20(*S*, *R*)-protopanaxadiol [PPD(*S*, *R*)] and 20(*S*, *R*)-protopanaxatriol [PPT(*S*, *R*)] are the structural basis response to a series of pharmacological effects of their parent components. Although the estrogenicity of several ginsenosides has been confirmed, however, the underlying mechanisms of their estrogenic effects are still largely unclear. In this work, PPD(*S*, *R*) and PPT(*S*, *R*) were assessed for their ability to bind and activate human estrogen receptor α (hERα) by a combination of *in vitro* and *in silico* analysis.

Methods: The recombinant hERα ligand-binding domain (hERα-LBD) was expressed in *E. coli* strain. The direct binding interactions of ginsenosides with hERα-LBD and their ERα agonistic potency were investigated by fluorescence polarization and reporter gene assays, respectively. Then, molecular dynamics simulations were carried out to simulate the binding modes between ginsenosides and hERα-LBD to reveal the structural basis for their agonist activities toward receptor.

Results: Fluorescence polarization assay revealed that PPD(*S*, *R*) and PPT(*S*, *R*) could bind to hERα-LBD with moderate affinities. In the dual luciferase reporter assay using transiently transfected MCF-7 cells, PPD(*S*, *R*) and PPT(*S*, *R*) acted as agonists of hERα. Molecular docking results showed that these ginsenosides adopted an agonist conformation in the flexible hydrophobic ligand-binding pocket. The stereostructure of C-20 hydroxyl group and the presence of C-6 hydroxyl group exerted significant influence on the hydrogen bond network and steric hindrance, respectively.

Conclusion: This work may provide insight into the chemical and pharmacological screening of novel therapeutic agents from ginsenosides.

1. Introduction

Panax ginseng is a well-known herb in the oriental countries and has been widely consumed in healthy food and as traditional medicine for thousands of years [1]. Previous studies have confirmed its extensive pharmacological effects on the cardiovascular, reproductive, and nervous systems [2,3]. Based on the increasing scientific evidences, it has been used for preventing and treating diseases such as diabetes, cancer, *etc* [4]. The therapeutic effects of *P. ginseng* can be attributed to a sort of compounds named ginsenosides, chemically described as ginseng saponins as well. Regarded as the foremost bioactive components in *P. ginseng*, ginsenosides have been discovered and identified for more than 180 species [5].

Because ginsenosides possess a common four-ring hydrophobic steroid-like structure with sugar moieties attached at C-3, C-6 or C-20 position, they can be divided into four subtypes as protopanaxadiol (PPD), protopanaxatriol (PPT), oleanolic acid, and octillol [6]. Owing to the diversity of chemical structure, the naturally occurring saponins exhibit a wide range of polarity and hydrophobicity, resulting in their distinct biological activities [7]. As the main metabolites of ginsenosides, 20(*S*, *R*)-protopanaxadiol [PPD(*S*, *R*)] and 20(*S*, *R*)-protopanaxatriol [PPT(*S*, *R*)] are the structural basis response to a series of pharmacological effects of their

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https://doi.org/10.1016/j.jgr.2018.05.001
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parent components [8]. Different orientations of the hydroxyl groups at C-20 provide stereoisomers, (*R*)-epimer and (*S*)-epimer, as shown in Fig. 1.

Based on the structural similarity of ginsenosides to steroid hormones, some pharmacological effects of ginsenosides may be mediated by binding to nuclear receptors, such as androgen receptor, estrogen receptors (ERs), glucocorticoid receptor, peroxisome proliferator-activated receptor γ, and progesterone receptor [9–12]. Nuclear receptor superfamily is a group of transcription factors that can be activated by the functional ligands, including 17β-estradiol, dexamethasone, fatty acids, *etc* [13–15]. As the main targets of phytoestrogens, ERs have been reported for their ability of binding several ginsenosides, including Re and Rh_1 [9,16]. Nevertheless, Rb_1 was observed to activate both ERα and ERβ in the absence of receptor binding, indicating that the estrogenic effect of Rb_1 was mediated *via* a ligand-independent pathway [16]. In summary, ginsenosides might exert their estrogenic activities by either directly binding or indirectly activating ERs.

Although previous studies have confirmed the estrogenicity of several ginsenosides, however, the underlying mechanisms of their estrogenic effects are still largely unclear. In this work, PPD(*S*, *R*) and PPT(*S*, *R*), *in vivo* metabolites of ginsenosides, were assessed for their ability to bind and activate human estrogen receptor α (hERα). First, the recombinant hERα ligand-binding domain (hERα-LBD) was expressed in *E. coli* strain. The direct binding interactions of ginsenosides with hERα-LBD and their ERα agonistic potency were investigated by fluorescence polarization (FP) and reporter gene assays, respectively. Then, molecular docking was carried out to simulate the binding modes between ginsenosides and hERα-LBD in an attempt to reveal the structural basis for their agonist activities toward receptor protein.

2. Materials and methods

2.1. Materials

Coumestrol (CS), 17β-estradiol (E_2), dimethylsulfoxide, and isopropyl β-D-1-thiogalactopyranoside (IPTG) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and TCI (Tokyo, Japan). Lipofectamine 2,000 transfection reagent, penicillin, and streptomycin were purchased from Invitrogen (Carlsbad, CA, USA). Dulbecco's modified Eagle's medium, and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Gibco BRL (Grand Island, NY, USA). Fetal bovine serum was purchased from HyClone (Logan, UT, USA). Ginsenosides PPD(*S*, *R*) and PPT(*S*, *R*) were purchased from Yuanye Biotechnology Co., Ltd. (Shanghai, China). The structures of the ginsenosides are shown in Fig. 1. All other reagents used were of analytical grade.

2.2. Preparation of glutathione S-transferase–hERα-LBD

The recombinant hERα-LBD (residues 282 to 595) was expressed as a fusion protein from the glutathione S-transferase (GST)-modified pGEX-4T-1 vector in *Escherichia coli* strain BL21(DE3)

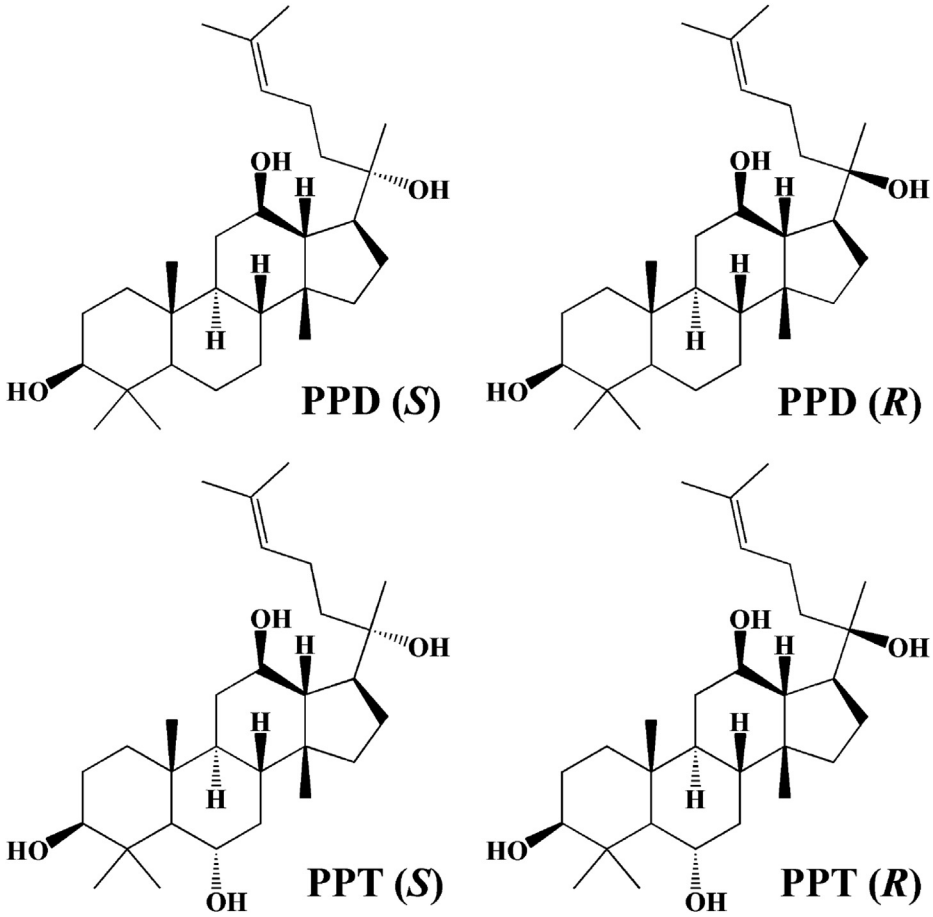

Fig. 1. Structures of 20(*S*, *R*)-protopanaxadiol [PPD(*S*, *R*)] and 20(*S*, *R*)-protopanaxatriol [PPT(*S*, *R*)] investigated in this work. Different orientations of the hydroxyl groups at C-20 provide stereoisomers, (*R*)-epimer and (*S*)-epimer.

pLysS. The coding region was synthesized *de novo* and inserted into BamHI and XhoI restriction enzyme sites. The plasmid pGEX-4T-1-hERα-LBD was introduced into BL21(DE3)pLysS. Expression of the GST-tagged hERα-LBD protein was induced with 0.5 mM IPTG overnight at 20°C. The supernatant was loaded onto a glutathione-Sepharose affinity column. Homogeneity of the purified protein was confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis.

2.3. Fluorescence polarization assay

The FP assay was performed by FlexStation 3 microplate reader (Molecular Devices, Sunnyvale, CA, USA) with excitation at 355 nm and emission at 405 nm. The dissociation constant ($K_{d,CS}$) of CS with hERα-LBD was obtained from the saturable binding experiment. Subsequently, in the competitive binding assay, hERα-LBD (250 nM) and CS (10 nM) were mixed in a total volume of 290 μL and then various concentrations of ginsenoside (10 μL) were added. Each sample was subjected to the FP assay after being incubated for 2 h at room temperature. If the added ginsenoside could compete with the tracer for the protein binding site, it would displace CS from CS–hERα-LBD complex, resulting in a low polarization value. The decrease of polarization values upon addition of competitive compound was monitored and plotted as a function of the concentrations of ginsenoside.

The half maximal inhibitory concentration (IC_{50}) value (the concentration of ginsenoside that inhibited the binding of CS with hERα-LBD by 50%) was calculated from the competition curves fitted using a four parameter logistic equation $Y = (A-D)/[1 + (X/IC_{50})^B] + D$, where Y and X correspond to the polarization value and the ginsenoside concentration, A and D are the polarization values at zero and an infinite concentration respectively, and B is the slope parameter [17]. The dissociation constant (K_d) of ginsenoside with hERα-LBD was calculated according to the relationship $IC_{50}/[\text{coumestrol}] = K_d/K_{d,CS}$. Data analysis was performed using GraphPad Prism 5 (GraphPad Software, USA).

2.4. Construction of plasmids

The coding region for full-length hERα was obtained by polymerase chain reaction–based accurate synthesis and then cloned into the pcDNA3.1(+) vector at restriction sites BamHI and XhoI to generate an expression plasmid pcDNA3.1(+)-hERα. The estrogen response element–luciferase (ERE-luc) reporter plasmid was constructed using a modified vector pGL6-CMV-TA-Luc containing the coding sequence for firefly luciferase. Two tandem repeats of the consensus ERE oligonucleotide (GTCAGGTCACAGTGACCTGAT) upstream of the minimal TA promoter were inserted into the BmtI-NdeI site. The control plasmid pRL-TK was purchased from Promega (Madison, WI, USA).

2.5. Cell proliferation and cytotoxicity assay

MCF-7 breast cancer cells preserved in our lab were routinely cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, 100 U/mL penicillin, and 100 μM streptomycin. Cells were maintained at 37°C in a humidified 5% CO_2 atmosphere. The cytotoxicity of each ginsenoside was measured using MTT assay. MCF-7 cells were seeded in a 96-well culture plate at a density of 1×10^4 cells/well and allowed to acclimatize for 12 h, then treated with different concentrations of ginsenosides for 24 h. At the end of treatment, 20 μL of MTT was added and incubated for an additional 4 h. The formazan crystals were dissolved in 200 μL of dimethylsulfoxide. After shaking the plate for 10 min, cell viability was assessed by measuring the absorbance at 550 nm.

2.6. Luciferase reporter assay

A mixture of pcDNA3.1(+)-hERα, ERE-luc, and pRL-TK plasmids were prepared at a ratio of 1:10:1 firstly. After exposure to the transfection reagents and plasmids for 12 h, the cells were washed with phosphate buffered saline (PBS) and then incubated with a medium containing different concentrations of ginsenosides for an additional 24 h. The activities of both firefly and Renilla luciferase in cell lysates were determined using a Dual-Glo Luciferase Assay System (Promega, Madison, WI, USA) according to the manufacturer's instruction on a Tecan Infinite F200 PRO microplate reader. Firefly luciferase activity was normalized against that of Renilla in three independent experiments. Data analysis was performed using GraphPad Prism 5 (GraphPad Software, USA).

2.7. Molecular dynamics simulations

The crystal structure of hERα-LBD in complex with *ortho*-trifluoromethylphenylvinyl estradiol (EZT) was available in Protein Data Bank (PDB ID: 2P15) [18]. The initial structures of PPD(*S*, *R*) and PPT(*S*, *R*) were constructed using GaussView and optimized with Gaussian 09 using the B3LYP/6-31G(d) method. The molecular length and Connolly solvent-excluded volume of the tested compounds were calculated using AutoDockTools-1.5.6 and Chem3D Ultra 8.0, respectively. Automated docking calculations were carried out by AutoDockTools-1.5.6 to explore the binding modes between hERα-LBD and these compounds. The grid box was generated at the center of the active site. The size of the grid box should be adjusted according to the length of each compound. Based on the scoring function of AutoDockTools-1.5.6, the predicted binding energies (kcal mol^{-1}) were calculated. For each compound, 10 independent docking runs were performed, and the one with the lowest binding energy was chosen for analysis. The intermolecular interactions between hERα-LBD and ligands were visualized with the program PyMol.

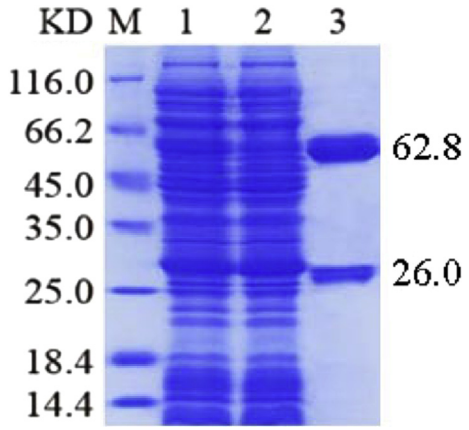

Fig. 2. SDS-PAGE analysis of GST–hERα-LBD fusion protein. M, molecular weight marker; lane 1, total protein; lane 2, flow-through (unbound); lane 3, eluate (bound). hERα-LBD, human estrogen receptor α–ligand-binding domain; GST, glutathione S-transferase.

Table 1
IC_{50} values, dissociation constants (K_d), and binding energies for PPD (*S*, *R*) and PPT (*S*, *R*)

Compound	IC_{50} (μM)	K_d (μM)	Binding energy (kcal mol^{-1})
PPD (*S*)	49.34	1260.64	−11.68
PPD (*R*)	54.49	1392.22	−11.51
PPT (*S*)	58.65	1498.51	−11.26
PPT (*R*)	67.17	1716.19	−11.12

PPD (*S*, *R*), 20(*S*, *R*)-protopanaxadiol; PPT (*S*, *R*), 20(*S*, *R*)-protopanaxatriol.

3. Results and discussion

3.1. Characterization of the recombinant protein

For the subsequent FP assay, a soluble form of the hERα-LBD protein should be produced first. In this work, the recombinant protein was expressed in the IPTG-induced *E. coli* cultures and then purified by affinity chromatography on glutathione-Sepharose. As shown in Fig. 2, a soluble protein with an apparent molecular mass of 62.8 kDa was achieved, corresponding to the GST fusion protein containing the ligand binding domain of hERα. Unfortunately, an additional band (26.0 kDa) migrating below GST−hERα-LBD was observed in SDS-PAGE analysis, suggesting that truncation of the GST tag might happen during overexpression of the recombinant protein in BL21(DE3)pLysS. As GST protein does not bind to ER [19], it can be speculated that the truncated tag may exert a negligible influence upon the ligand-ER interaction. Hence, although the soluble proteins produced in this work contain both intact GST-hERα-LBD and truncated GST, they are still suitable for *in vitro* binding assay.

3.2. Assessment of ginsenosides binding potency with hERα-LBD

Estrogen mimetics such as xenoestrogens and phytoestrogens regulate physiological functions in target tissues primarily by binding to steroid hormone receptors [20]. To test whether PPD(*S*, *R*) and PPT(*S*, *R*) mediate their biological effects through direct binding to hERα-LBD, the competitive binding assay was carried out by FP. CS, a plant-derived autofluorescent compound, was chose as tracer for the ligand displacement experiment. It can compete with physiological estrogen (17β-estradiol) for binding to ER as well as stimulate the activity of reporter genes in the presence of its receptor protein [21]. As can be seen in Fig. 3, all the tested ginsenosides exhibited dose-dependent binding to hERα-LBD. Base on the $K_{d,CS}$ of 255.50 nM determined in our previous work, the IC_{50} values and dissociation constants (K_d) of hERα-LBD

Table 2
The molecular length, Connolly solvent-excluded volume (CSEV), and docking results of the tested compounds to human estrogen receptor α

Compound	Length (Å)	CSEV (Å^3)	Hydrogen bonds	Estrogenicity
EZT	11.782	391.5	Glu353, Leu387, Arg394, His524, H_2O	Agonist
PPD(*S*)	14.709	483.2	Glu353	Agonist
PPD(*R*)	15.265	483.9	Glu353, His524	Agonist
PPT(*S*)	14.948	488.7	Glu353	Agonist
PPT(*R*)	15.258	489.6	Glu353, His524	Agonist

EZT, *ortho*-trifluoromethylphenylvinyl estradiol; PPD, protopanaxadiol; PPT, protopanaxatriol.

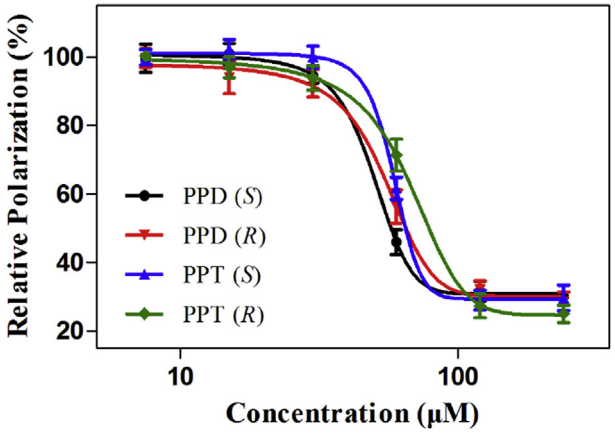

Fig. 3. Competitive binding of PPD(*S*, *R*) and PPT(*S*, *R*) to hERα-LBD. Results are given as means ± SEM of three independent experiments.
hERα-LBD, human estrogen receptor α−ligand-binding domain; PPD (*S*, *R*), 20(*S*, *R*)-protopanaxadiol; PPT (*S*, *R*), 20(*S*, *R*)-protopanaxatriol.
SEM, standard error of the mean

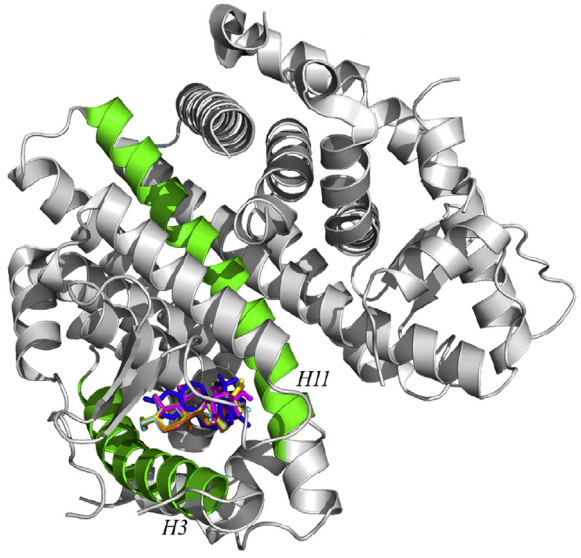

Fig. 5. Superimposition of the docking poses of different ligands in the hERα-LBD. EZT, blue; PPD(*S*), yellow; PPD(*R*), cyan; PPT(*S*), magenta; PPT(*R*), orange.
hERα-LBD, human estrogen receptor α−ligand-binding domain; PPD, protopanaxadiol; PPT, protopanaxatriol.

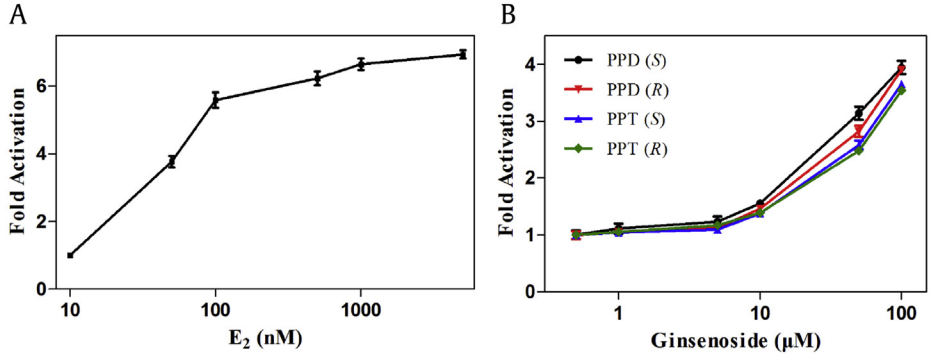

Fig. 4. The hERα activation potency determined by ERE-luciferase reporter gene assay in MCF-7 cells. (A) Of E_2. (B) Of ginsenosides. Results are given as means ± SD of three independent experiments.
ERE, estrogen response element; hERα, human estrogen receptor α; PPD, protopanaxadiol; PPT, protopanaxatriol; SD, standard deviation.

for PPD(*S*, *R*) and PPT(*S*, *R*) were measured and illustrated in Table 1. The K_d values of the tested ginsenosides are in the range of 1260.64 μM to 1716.19 μM, reflecting their moderate affinities for hERα-LBD. With respect to all these compounds, the binding affinities are in the order of PPD(*S*) > PPD(*R*) > PPT(*S*) > PPT(*R*). In summary, all the ginsenosides investigated in this work can bind to hERα-LBD as the functional ligands, resulting in the activation of ER which may in turn influence the regulation of various physiological functions.

3.3. Biological effect of ginsenosides on hERα activity

For subsequent luciferase reporter gene assay, the cytotoxicity of the tested compounds should be assessed by MTT assay first. The hERα activity in MCF-7 cells was measured under the exposure of noncytotoxic concentrations of ginsenosides. This allowed us to ensure that changes in hERα activity were due to ginsenosides treatment but not a secondary effect of cell death. In the present work, MCF-7 cells were transiently cotransfected with plasmids of

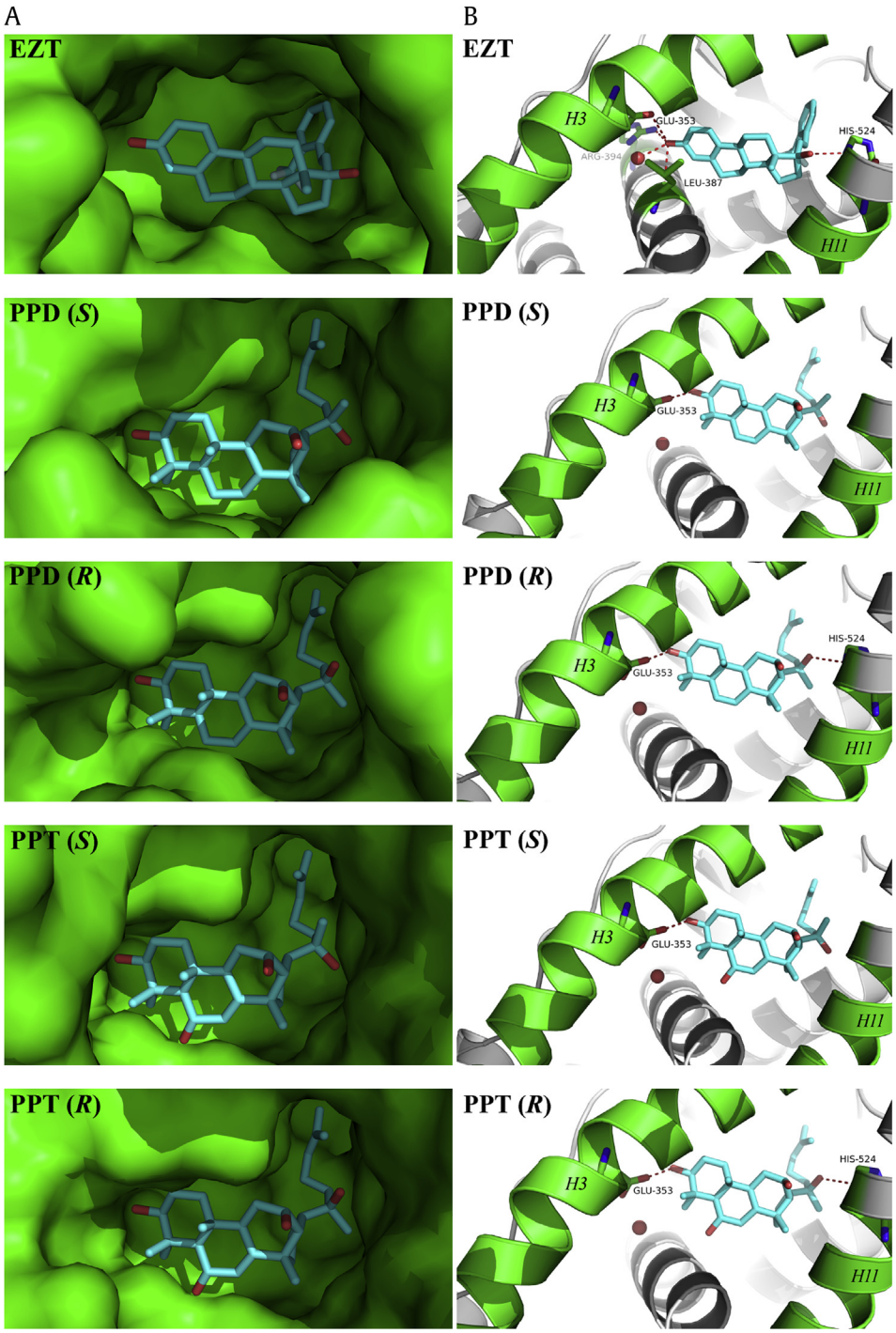

Fig. 6. Computational docking of EZT, PPD(*S*, *R*) and PPT(*S*, *R*) to hERα-LBD. (A) The hydrophobic binding pocket of hERα-LBD to stabilize ligands. (B) The profiles of ligand-hERα-LBD interaction. Red sphere, water molecule; red dashed lines, hydrogen bonds.
hERα-LBD, human estrogen receptor α–ligand-binding domain; EZT, ortho-trifluoromethylphenylvinyl estradiol; PPD, protopanaxadiol; PPT, protopanaxatriol.

pcDNA3.1(+)-hERα and ERE-luc to investigate the activation effect of ginsenosides on the hERα pathway. The cells were also cotransfected with a plasmid (pRL-TK) encoding Renilla luciferase controlled by a constitutively active promoter to correct for the difference in both transfection and harvest efficiencies between experiments. The fold of hERα activation was expressed as the ratio of firefly to Renilla luciferase activity (Fluc/Rluc). The transfected cells were treated with the tested compounds over a range of concentrations. After incubation for 24 h, luciferase activity was measured.

As shown in Fig. 4A, the *in vitro* transient transfection system in MCF-7 cells is well established and suitable for evaluating the effects of ginsenosides on hERα activation. Luciferase reporter gene assay showed that hERα was activated by PPD(*S*, *R*) and PPT(*S*, *R*) in a dose-dependent manner with a maximal of four-fold increase (Fig. 4B). Furthermore, it can be observed that the order of activation potency for ginsenosides is in good agreement with their binding affinities with hERα-LBD.

3.4. Structural basis for ginsenosides binding with hERα-LBD

Major ginsenosides are a series of dammarane-type triterpene homologes that share a common hydrophobic four-ring steroid-like structure. They can be categorized into two groups according to the aglycone structure: PPD and PPT types. The former has hydrophilic sugar moieties attached at the positions of C-3 and/or C-20 (such as Rb_1, Rb_2, Rc, and Rd), while the latter has hydrophilic sugar moieties attached at the positions of C-3, C-6, and/or C-20 (such as Re and Rg_1) [22,23]. As the main metabolites of ginsenosides, PPD(*S*, *R*) and PPT(*S*, *R*) were observed to bind and activate hERα in this work. Then, to elucidate the structure-activity relationship with a focus on hydroxyl groups located in different positions and the stereoselectivity of 20(*S*) and 20(*R*), the binding modes between ginsenosides and hERα-LBD were simulated by molecular docking.

Based on the crystal structure of human ERα in complex with 17β-estradiol (E_2) [24], the ligand-binding pocket (LBP) with a probe accessible volume of approximately 450 $Å^3$ can hardly accommodate PPD(*S*, *R*) or PPT(*S*, *R*) (~485 $Å^3$), as summarized in Table 2. With regard to this, the ERα-EZT structure with a novel extended pocket was employed for molecular dynamics simulations. In contrast with previous ER structures, the phenylvinyl substitution of EZT increases the volume of the cavity by 40% through remodeling of helix 7 into an extended loop [18], making the LBP large enough to encapsulate PPD(*S*, *R*) or PPT(*S*, *R*). As shown in Fig. 5 and Table 2, the skeleton length of ginsenosides (~15 Å) is well matched by the LBP. They completely fit into the cavity without disrupting the coactivator binding site, known as a transcriptional activation function (AF-2). Interestingly, PPD(*S*, *R*) and PPT(*S*, *R*) all adopt an agonist conformation similar to that of EZT (Fig. 5), a potent agonist ligand.

Molecular docking results suggested that the stereostructure of the C-20 hydroxyl group exerts significant influence on the hydrogen bonding and hydrophobic interactions. As shown in Fig. 6, 20(*R*)-ginsenosides form a hydrogen bond with His524 in H11. However, the hydrogen bond between this amino acid site and 20(*S*)-ginsenosides has not been observed. Thus, the higher affinities of 20(*S*)-ginsenosides may be associated with an increase in the number of hydrophobic contacts compared with their 20(*R*) counterparts. Besides, the C-3 hydroxyl group locates in the active site of hERα-LBD and makes a hydrogen bond interaction with Glu353 in H3. On the other hand, with a hydroxyl group at C-6, the steric hindrance for PPT to bind to hERα-LBD increases, and thus significantly reducing their binding potency compared to PPD, as shown in Table 1. It has been reported that the PPD-type ginsenosides generally exert more potent biological effects than those of the PPT-type [25], which is confirmed in this work.

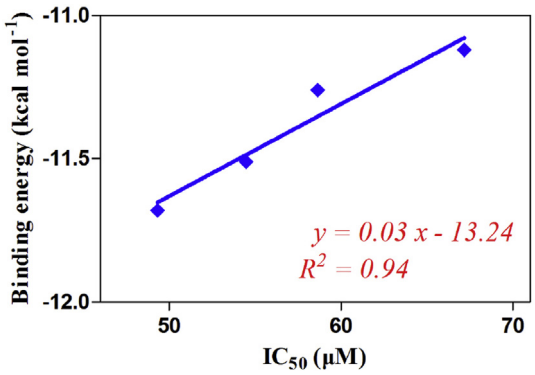

Fig. 7. Correlation of the calculated binding energies to the determined binding affinities for PPD(*S*, *R*) and PPT(*S*, *R*).
PPD (*S*, *R*), 20(*S*, *R*)-protopanaxadiol; PPT (*S*, *R*), 20(*S*, *R*)-protopanaxatriol.

Furthermore, the order of the calculated binding potency for ginsenosides with hERα-LBD is consistent with their experimentally determined binding affinities (Table 1). As shown in Fig. 7, comparison of the docking scores versus the IC_{50} values yields a good correlation (R^2 = 0.94), indicating that molecular dynamics simulations can potentially be applied for predicting the binding potency of novel bioactive compounds toward target receptors.

4. Conclusion

In this work, the relationship between ginsenoside structures and their estrogenicity was assessed by a combination of *in vitro* and *in silico* analysis. ERE-luc reporter gene assay showed that PPD(*S*, *R*) and PPT(*S*, *R*) were capable of activating hERα in transiently transfected MCF-7 cells. FP competitive binding assay suggested that these ginsenosides exert estrogenic activities through binding to hERα-LBD. Furthermore, molecular dynamics simulations were performed to elucidate the underlying mechanisms of their estrogenic effects. In conclusion, as the main metabolites of ginsenosides, PPD(*S*, *R*) and PPT(*S*, *R*) display apparent functional properties comparable to those of ER agonists. This work may provide insight into chemical and pharmacological approaches in the discovery and evaluation of novel therapeutic agents that target ER.

Conflicts of interest

All authors have no conflicts of interest to declare.

Acknowledgments

This work was supported by the Science and Technology Development Project Foundation of Jilin Province (20180520102JH and 20180201062SF), the China Postdoctoral Science Foundation (2017M621213), the National Key Research and Development Program of China (2017YFD0300603 and 2016YFD0300103), and the National Natural Science Foundation of China (31601534 and 31701349).

References

[1] Heng Y, Zhang Q-S, Mu Z, Hu J-F, Yuan Y-H, Chen N-H. Ginsenoside Rg1 attenuates motor impairment and neuroinflammation in the MPTP-probenecid-induced parkinsonism mouse model by targeting α-synuclein abnormalities in the substantia nigra. Toxicol Lett 2016;243:7–21.

[2] Lee CH, Kim J-H. A review on the medicinal potentials of ginseng and ginsenosides on cardiovascular diseases. J Ginseng Res 2014;38:161–6.
[3] Vo HT, Cho JY, Choi Y-E, Choi Y-S, Jeong Y-H. Kinetic study for the optimization of ginsenoside Rg3 production by heat treatment of ginsenoside Rb1. J Ginseng Res 2015;39:304–13.
[4] Lee Y-M, Yoon H, Park H-M, Song BC, Yeum K-J. Implications of red Panax ginseng in oxidative stress associated chronic diseases. J Ginseng Res 2017;41:113–9.
[5] Khan Chowdhury E, Jeon J, Ok Rim S, Park Y-H, Kyu Lee S, Bae H. Composition, diversity and bioactivity of culturable bacterial endophytes in mountain-cultivated ginseng in Korea. Sci Rep 2017;7:10098.
[6] Park SM, Jung EH, Kim JK, Jegal KH, Park CA, Cho IJ, Kim SC. 20S-Protopanaxadiol, an aglycosylated ginsenoside metabolite, induces hepatic stellate cell apoptosis through liver kinase B1–AMP-activated protein kinase activation. J Ginseng Res 2017;41:392–402.
[7] Chen RJY, Chung T-y, Li F-y, Lin N-h, Tzen JTC. Effect of sugar positions in ginsenosides and their inhibitory potency on Na+/K+-ATPase activity. Acta Pharmacol Sin 2009;30:61–9.
[8] Zhao X-E, Lv T, Zhu S, Qu F, Chen G, He Y, Wei N, Li G, Xia L, Sun Z, et al. Dual ultrasonic-assisted dispersive liquid-liquid microextraction coupled with microwave-assisted derivatization for simultaneous determination of 20(S)-protopanaxadiol and 20(S)-protopanaxatriol by ultra high performance liquid chromatography-tandem mass spectrometry. J Chromatogr A 2016;1437:49–57.
[9] Furukawa T, Bai C-X, Kaihara A, Ozaki E, Kawano T, Nakaya Y, Awais M, Sato M, Umezawa Y, Kurokawa J. Ginsenoside Re, a main phytosterol of Panax ginseng, activates cardiac potassium channels via a nongenomic pathway of sex hormones. Mol Pharmacol 2006;70:1916–24.
[10] Lee YJ, Chung E, Lee KY, Lee YH, Huh B, Lee SK. Ginsenoside-Rg1, one of the major active molecules from Panax ginseng, is a functional ligand of glucocorticoid receptor. Mol Cell Endocrinol 1997;133:135–40.
[11] Leung KW, Cheung LWT, Pon YL, Wong RNS, Mak NK, Fan TPD, Au SCL, Tombran-Tink J, Wong AST. Ginsenoside Rb1 inhibits tube-like structure formation of endothelial cells by regulating pigment epithelium-derived factor through the oestrogen β receptor. Br J Pharmacol 2007;152:207–15.
[12] Zhang Y, Yu L, Cai W, Fan S, Feng L, Ji G, Huang C. Protopanaxatriol, a novel PPARγ antagonist from Panax ginseng, alleviates steatosis in mice. Sci Rep 2014;4:7375.
[13] Rataj F, Möller FJ, Jähne M, Zierau O, Diel P, Vollmer G, Kretzschmar G. Regulation of uterine AHR battery gene expression by 17β-Estradiol is predominantly mediated by estrogen receptor α. Arch Toxicol 2012;86:1603–12.
[14] Zhang J, Xing X, Sun Y, Li Z, Xue P, Wang T, Li T. Characterization of the binding between phthalate esters and mouse PPARα for the development of a fluorescence polarization-based competitive binding assay. Anal Methods 2016;8:880–5.
[15] Zhang J, Zhang T, Guan T, Yu H, Li T. In vitro and in silico assessment of the structure-dependent binding of bisphenol analogues to glucocorticoid receptor. Anal Bioanal Chem 2017;409:2239–46.
[16] Cho J, Park W, Lee S, Ahn W, Lee Y. Ginsenoside-Rb1 from Panax ginseng C.A. Meyer activates estrogen receptor-α and -β, independent of ligand binding. J Clin Endocrinol Metab 2004;89:3510–5.
[17] Zhang J, Zhang T, Guan T, Ruan P, Ren D, Dai W, Yu H, Li T. Spectroscopic and molecular modeling approaches to investigate the interaction of bisphenol A, bisphenol F and their diglycidyl ethers with PPARα. Chemosphere 2017;180:253–8.
[18] Nettles KW, Bruning JB, Gil G, O'Neill EE, Nowak J, Guo Y, Kim Y, DeSombre ER, Dilis R, Hanson RN, et al. Structural plasticity in the oestrogen receptor ligand-binding domain. EMBO Rep 2007;8:563–8.
[19] Hong H, Kohli K, Trivedi A, Johnson DL, Stallcup MR. GRIP1, a novel mouse protein that serves as a transcriptional coactivator in yeast for the hormone binding domains of steroid receptors. Proc Natl Acad Sci USA 1996;93:4948–52.
[20] Watson CS, Bulayeva NN, Wozniak AL, Finnerty CC. Signaling from the membrane via membrane estrogen receptor-α: estrogens, xenoestrogens, and phytoestrogens. Steroids 2005;70:364–71.
[21] Kuiper GG, Carlsson B, Grandien K, Enmark E, Häggblad J, Nilsson S, Gustafsson JA. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β. Endocrinology 1997;138:863–70.
[22] Han J-Y, Kim H-J, Kwon Y-S, Choi Y-E. The Cyt P450 enzyme CYP716A47 catalyzes the formation of protopanaxadiol from dammarenediol-II during ginsenoside biosynthesis in Panax ginseng. Plant Cell Physiol 2011;52:2062–73.
[23] Kim D-H. Chemical diversity of Panax ginseng, Panax quinquifolium, and Panax notoginseng. J Ginseng Res 2012;36:1–15.
[24] Brzozowski AM, Pike AC, Dauter Z, Hubbard RE, Bonn T, Engström O, Ohman L, Greene GL, Gustafsson JA, Carlquist M. Molecular basis of agonism and antagonism in the oestrogen receptor. Nature 1997;389:753–8.
[25] Wang C-Z, Anderson S, Du W, He T-C, Yuan C-S. Red ginseng and cancer treatment. Chin J Nat Med 2016;14:7–16.