Biochemical and Biophysical Research Communications xxx (2016) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Interaction of human chymase with ginkgolides, terpene trilactones of *Ginkgo biloba* investigated by molecular docking simulations

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ARTICLE INFO

Article history:
Received 16 February 2016
Accepted 8 March 2016
Available online xxx

Keywords:
Ginkgo biloba
Chymase
Herbal nutraceutical
Screening
Molecular docking
Inhibitor
Cardiovascular diseases

ABSTRACT

The search for natural chymase inhibitors has a good potential to provide a novel therapeutic approach against the cardiovascular diseases and other heart ailments. We selected from literature 20 promising *Ginkgo biloba* compounds, and tested them for their potential ability to bind chymase enzyme using docking and a deep analysis of surface pocket features. Docking results indicated that the compounds may interact with the active site of human chymase, with favorable distinct interactions with important residues Lys40, His57, Lys192, Phe191, Val146, Ser218, Gly216, and Ser195. In particular, proanthocyanidin is the one with the best-predicted binding energy, with seven hydrogen bonds. Interestingly, all active *G. biloba* compounds have formed the hydrogen bond interactions with the positively charged Lys192 residue at the active site, involved in the mechanism of pH enhancement for the cleavage of angiotensin I site. Ginkgolic acid and proanthocyanidin have better predicted binding energy towards chymase than other serine proteases, i.e kallikrein, tryptase and elastase, suggesting specificity for chymase inhibition. Our study suggests these *G. biloba* compounds are a promising starting point for developing chymase inhibitors for the potential development of future drugs.

1. Introduction

Cardiovascular disease is becoming a global phenomenon all over the world, and one particular manifestation, heart failure, is perilously increasing in regularity. The quantity of cardiac mast cells is remarkably increased in patients with heart failures, and a strong connection between heart failure and chymase (EC 3.4.21.39), an important enzyme present in abundance in secretory granules of mast cells, has been already proved [2]. Chymase is accumulated in mast cells in an inactive form and it is activated suddenly after its release into the interstitial tissues at pH 7.4, in particular when mast cells are stimulated by inflammation or injury. This enzyme is the extensive extravascular source of vasoactive angiotensin II, which is formed very rapidly through the hydrolysis of the Phe8–His9 bond of angiotensin I [8]. For these reasons, there is a strong interest to develop specific chymase inhibitors [3], as a new therapeutic treatment for the disease as well as a potential therapeutic modality for atherosclerotic plaque stabilization.

Ginkgo biloba (Ginkgoaceae) (GB), also known as 'maidenhair tree', is the best-selling herbal remedy in the USA [31]. GB extracts have two major fractions of compounds: flavonoids and terpenes. The chemical structure of flavonoids comprises an aromatic ring and a double bond that seem to react preferentially with hydroxyl radicals [35]. Terpenes comprise the ginkgolides A, B, C, J and M, and bilobalide [27]. Terpenes were found to strongly diminish platelet activation and collection by antagonizing platelet-activating factor [28,20], whereas bilobalide, a sesquiterpene-trilactone, was shown to reduce cerebral edema, cortical infarct volume and ischemic damage in patients who have suffered a stroke [9]. Moreover, an extract of GB was proven to reduce the atherosclerotic nanoplaque formation after a 2-months therapy. This effect was attributed to an up-regulation of radical scavenging enzymes and the inhibition of the risk factors, oxidized Low Density Lipoprotein/Low Density Lipoprotein and Lipoprotein [26]. Furthermore, *in vitro* experiments on a rat model of obese type 2

Abbreviations: GB, *Ginkgo biloba*; OHH, 2-[3-{methyl [1-(2-naphthoyl) piperidin-4-yl] amino} carbonyl)-2-naphthyl]-1-(1-naphthyl)-2-oxoethylphosphonic acid; PDB, Protein Data Bank.

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http://dx.doi.org/10.1016/j.bbrc.2016.03.028
0006-291X/

diabetes show that GB extract has beneficial effects on blood circulation and hyperglycemia, and provides protective effect in patients with diabetes and atherosclerosis [17].

In our *in silico* study, we tested components of GB extracts for their ability to bind to human chymase enzyme. Since chymase is also involved in atherosclerosis [7], we would like to challenge the hypothesis of a correlation between them.

2. Methods

2.1. Selection of compounds

Twenty natural compounds from GB were selected from literature [6,15,23,24,29,32] (see Fig. S1 for their chemical structures). PubChem [14] was used to retrieve all ligand molecules in the SDF format. Their 3D structures were then converted to Protein Data Bank (PDB) format using Chimera program [22].

PDB [4] includes 18 human chymase structures, and they were compared and analyzed for resolution, reliability and Ramachandran plot with PDB Redo [13]. The crystal structure of chymase complexed with the ligand JNJ-10311795 (2-[3-{methyl [1-(2-naphthoyl) piperidin-4-yl] amino} carbonyl)-2-naphthyl]-1-(1-naphthyl)-2-oxoethylphosphonic acid) (OHH) (PDB code 1T31) [10] has been selected as the best one in terms of completeness and quality. Two known natural chymase inhibitors, i.e. keto-beta-boswellic acid and beta-boswellic acid [30], were downloaded from PubChem database and used as control for molecular docking simulations.

2.2. Molecular docking simulation of GB compounds

Protein–ligand docking simulations were performed using AutoDock version 4.2 and ADT Suite 1.5.6 software [19]. The structure of the crystallographic inhibitor OHH bound to chymase was used as a control to perform a self-docking prediction, to check for correctness of the parameters as well as for estimating its binding energy. Furthermore, in order to check for selectivity of the GB compounds towards chymase, other proteins sharing the same enzymatic activity were selected, namely kallikrein (PDB code: 1LO6) [5], tryptase (PDB code: 2FPZ) [18], elastase (PDB code: 5ABW) [33], and docking simulations were performed. Polar hydrogens were added to the protein and ligands, and charges were assigned according to Gasteiger [12]. A box of 76 × 60 × 74 points was used for all docking simulations towards chymase, with a spacing of 0.353 Å, targeting the pool of amino acids Lys40, His57, Asp102, Phe191, Lys192, Gly193, Ser195, Tyr215, Gly216, Arg217, Ala226 identified in the PDB file as the ones involved in the interaction with the ligand. For docking towards kallikrein, a box of 64 × 66 × 72 points was used, with a spacing of 0.353 Å, centered on the pool of amino acids involved in the interaction with the ligand (as reported in the 1LO6.pdb file), i.e. His57, Asp189, Ser190, Cys191, Gln192, Gly193, Asp194, Ser195, Val213, Ser214, Tyr215, Gly216, Asn217, Ile218, Cys220, Ser221, Pro225, Gly226, Tyr228. For tryptase, a box of 76 × 68 × 66 points was used, with a spacing of 0.353 Å, centered on the pool of amino acids involved in the interaction with the ligand (as reported in the 2FPZ.pdb file), i.e. His57, Tyr172, Ser190, Cys191, Gln192, Gly193, Asp194, Ser195, Val213, Tyr215, Gly216, Glu217, Gly219, Cys220, Ala221, Arg224, Pro225, Gly226, Ile227, Tyr228. For elastase, a box of 70 × 68 × 74 points was used, with a spacing of 0.353 Å, centered on the pool of amino acids involved in the interaction with the ligand (as reported in the 5ABW.pdb file), i.e. His57, Tyr94, Val97, Asn98, Leu99, Arg177, Val190, Cys191, Phe192, Gln192, Gly193, Asp194, Ser195, Gly196, Ala213, Ser214, Phe215, Val216, Arg217, Gly218, Cys220. Before docking, water molecules and the ligands present in the crystallographic structure were removed. For each GB compound, 100 docking runs were performed using the AutoDock Lamarckian genetic algorithm, considering the protein as rigid and the ligand as flexible. The others parameters were set to default. RMSD value of 2.0 Å was taken for clustering docking poses. The Discovery Studio software (Dassault Systèmes BIOVIA) was used for superimposing co-crystallized inhibitor of chymase and GB compounds to compare the position of binding.

The conformations illustrative of the best energetic and the most populated cluster of poses were selected, saved in .pdb format and investigated for their H-bonds and hydrophobic interactions with the enzyme by using the tools available in AutoDock Tools and Discovery Studio.

2.3. Chymase surface pocket analysis

CASTp server [16] was used with default parameters to identify the possible ligand binding pockets within the active site of the 3D structures of chymase.

3. Results

3.1. Molecular docking of G. biloba compounds with chymase

By molecular docking simulations, we found that all the 20 compounds derived from GB derivatives are predicted to bind to the active site of chymase, since their predicted binding energies are negative (see Table 1). In particular, on the basis of lowest predicted binding energy, the detailed study of the best four different GB compounds (highlighted in Table 1) predicted to bind to chymase showed that all of them interact with at least one of the key amino acids constituting the active site of human chymase, which are Lys40, His57, Asp102, Phe191, Lys192, Gly193, Ser195, Tyr215, Gly216, Arg217, Ala226 (Fig. 1). Their detailed interactions are reported in Supplementary Table 1.

In order to compare the results of the selected GB compounds with known natural chymase inhibitors, we performed molecular docking simulations with beta-boswellic acid and keto-beta-boswellic acid [30]. Moreover, we performed a re-docking procedure with OHH compound, co-crystallyzed with chymase in PDB structure 1T31. Results are shown in Table 2.

While the re-docking of OHH indicates very low binding energy, the values of predicted binding energy for the other two known chymase inhibitors are very similar to those obtained with ginkgolic acid, proanthocyanidins, quercetrin and rutin, supporting the hypothesis that the GB-derived compounds can indeed bind chymase. From this analysis, it appears that these best four GB compounds are predicted to interact with chymase key residues Lys40, His57, Phe191, Lys192, Ser195 and Gly216.

We performed further docking simulations to verify the possibility of interactions of the four GB compounds with other serine proteases, i.e, kallikrein, tryptase, elastase (Table 3). Ginkgolic acid and proanthocyanidin have better predicted binding energy towards chymase than towards kallikrein, tryptase and elastase. Therefore, these two compounds appear rather selective for chymase. On the contrary, quercitrin shows similar predicted binding energy for all proteins and therefore appears to be not selective. Finally the predicted binding energies for rutin indicate that this molecule appears to bind preferentially to kallikrein than to chymase, but less preferentially to tryptase and elastase.

3.2. Chymase surface analysis and comparison to other serine proteases

The chymase structure was analyzed by CASTp program to

Please cite this article in press as: A. Dubey, et al., Interaction of human chymase with ginkgolides, terpene trilactones of *Ginkgo biloba* investigated by molecular docking simulations, Biochemical and Biophysical Research Communications (2016), http://dx.doi.org/10.1016/j.bbrc.2016.03.028