Journal of Molecular Graphics and Modelling 80 (2018) 190–196

Contents lists available at ScienceDirect

Journal of Molecular Graphics and Modelling

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

Investigation of 15-hydroxyprostaglandin dehydrogenase catalytic reaction mechanism by molecular dynamics simulations

Belal O. Al-Najjar[a,b,*]

[a] Faculty of Pharmacy and Medical Sciences, Al-Ahliyya Amman University, Amman, Jordan
[b] Molecular Modeling and Drug Design lab, Al-Ahliyya Amman University, Amman, Jordan

ARTICLE INFO

Article history:
Received 11 November 2017
Received in revised form 14 January 2018
Accepted 15 January 2018

Keywords:
15-PGDH
Molecular dynamics simulation
Molecular docking
PGE_2
MM-GBSA

ABSTRACT

15-hydroxyprostaglandin dehydrogenase (15-PGDH) is a prostaglandin metabolizing enzyme that oxidizes the hydroxyl group at carbon 15 (C15). The aim of the present work is to propose the main amino acids that catalyze the reaction through studying the intermolecular interaction between the ligand and the enzyme inside the active site using molecular dynamics simulation (MD). Therefore, MD simulations for two 15-PGDH systems bound with a substrate (PGE_2) or an inhibitor (compound 4) were performed to investigate the importance of ligand interaction on the behavior of amino acids in the active site. Findings from this work proposed the amino acids: Tyr151, Gln148 & Asn95 to act as a catalytic triad for the reaction as hydrogen bond interactions, dihedral rotation analysis and MM-GBSA free energy calculations revealed.

1. Introduction

Arachidonic acids undergo biotransformation by cyclooxygenase (COX) enzymes, to produce different types of prostaglandins [1]. These prostaglandins are important to regulate several physiological actions such as homeostasis, reproduction, and immune response [2]. Prostaglandins are rapidly metabolized by the oxidation of its 15-hydroxyl group catalyzed by 15-hydroxyprostaglandin dehydrogenase (15-PGDH) [3]. This metabolizing enzyme can be found in various mammalian tissues mainly in the; lung, kidney and placenta [4]. Several studies showed that the inhibition of 15-PGDH enzyme may lead to a decrease in hair loss [5], peptic ulcer healing [6,7], bone formation [8] and interestingly the facilitation of skin wound healing [9,10].

In literature, several inhibitors of 15-PGDH were synthesized along with the identification of their biological activity [11–14]. However, only few molecular modeling studies such as, molecular docking and 3D-QSAR analysis were performed on the enzyme [15]. Pharmacophore modeling and 3D-QSAR studies were previously performed in our laboratory to establish valid models for virtual screening to find novel enzyme inhibitors [16] .It is worth to note that Hamza et al. have prepared a homology model of the enzyme to perform molecular dynamics simulation [17]. Nevertheless, it was not until 2010 that the first X-ray 15-PGDH enzyme has been published as a holoenzyme form. The X-ray structure was documented under the code 2GDZ in the Protein Data Bank [18]. In their work, Hamza et al. have proposed the ability of Tyr151 to subtract a proton at the C15 hydroxyl group of PGE_2 and transfer it to NAD^+ producing the metabolite 15-keto-PGE_2. They presented the involvement of Ser138 and Gln148 in the formation of hydrogen bonds at the C15 hydroxyl group of PGE_2.

The aim of the present work is to provide an updated insight on the intermolecular interaction between the ligand and the enzyme inside the active site using molecular dynamics simulation of the crystalized enzyme 2GDZ. Findings from this wok may help scientists better understand the binding mechanism thus helping them to design and develop potent and selective inhibitors. In this study, molecular dynamics simulations for two 15-PGDH systems were performed to investigate the importance of ligand interaction on the stabilization of the systems. Unlike previous studies which have worked on the homology model emphasizing on the importance of Glu148 [17] or Asn91, Gly93, Gly12 and Tyr151 [15] for the catalytic reaction, this study depended on the actual X-ray structure to predict the catalytic residues important for the reaction. Thereby, findings from the study could provide an updated valuable insight on the molecular interaction and specify the important interactions for the catalytic reaction.

* Corresponding author at: Faculty of Pharmacy and Medical Sciences, Al-Ahliyya Amman University, Amman, Jordan.
E-mail address: b.najjar@ammanu.edu.jo

https://doi.org/10.1016/j.jmgm.2018.01.012
1093-3263/

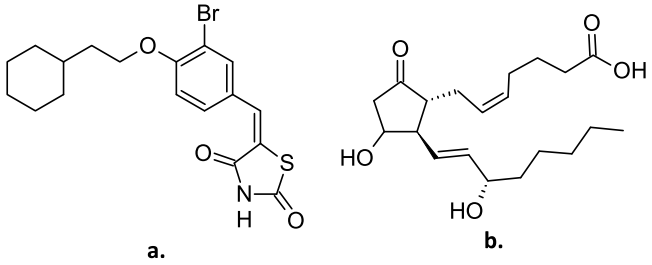

Fig. 1. 2D chemical structure of a. compound 4 as enzyme inhibitor and b. prostaglandin E_2 as an enzyme substrate.

2. Materials and methods

The crystal structure of 15-hydroxyprostaglandin dehydrogenase (PDB code 2GDZ) was obtained from the Protein Data Bank (www.rcsb.org/pdb) [18] to be used in this study. The structure is a holoenzyme complexed with NAD^+ cofactor which was used for both molecular docking and molecular dynamic simulations. Simulations performed in this study constituted of two binding ligands, one is prostaglandin E2 (PGE_2), a natural substrate of the enzyme, and the second is compound 4 reported by Ying Wu et al. (2011) as an inhibitor [19], Fig. 1. Ying Wu et al. have synthesized and studied the biological activity of several inhibitors. In their study, they reported compounds 3 and 4 as potent inhibitors with an IC_{50} of 8 and 19 nM, respectively. Nevertheless, *in vivo* studies from their work demonstrated a significant improvement of wound healing after the treatment of cells by compound 4 [19].

2.1. Molecular modeling software

The following software packages were used in the project:

a ACD/ChemSketch, (www.acdlabs.com) [20]
b Autodock 4.2 [21]
c AMBER 16 [22]
d Biovia Discovery Studio visualizer (http://accelrys.com)

2.2. Molecular docking

As yet, the available crystal structure of 15-PGDH in Protein Data Bank is a holoenzyme form. Molecular docking simulations of compound 4 and PGE_2 were performed on the protein 2GDZ. Both hydrogen atoms and atomic charges were added to the selected ligands and the enzyme. Kollman united atom charges were added to the enzyme, while Gasteiger charges were used for the selected ligands. The solvation parameters were then added by the ADDSOL utility found in AutoDock 4.2. Autogrid 4 utility was used to perform grid calculation with box dimension of 22.5 Å and grid spacing of 0.375 Å. Lamarckian genetic algorithm (LGA) was used for energy optimization and minimization during docking simulation.

2.3. Molecular dynamics

In this study two molecular dynamics simulations were performed using AMBER 16. For a timescale of 20 ns each, these simulations were run to study the key interactions in the active site for enzyme. These molecular dynamics simulations are denoted as follows:

i **ECS** = PGDH (**E**nzyme) + NAD^+ (**C**ofactor) + PGE_2 (**S**ubstrate)
ii **ECI** = PGDH (**E**nzyme) + NAD^+ (**C**ofactor) + compound 4 (**I**nhibitor)

2.3.1. Model setup

Starting model contained 666 amino acid residues of PGDH with NAD^+ present in the structure along with the docked ligand, either PGE_2 or compound 4, see Section 2.2. Molecular docking. Explicit hydrogen atoms were added utilizing LEAP module embedded in AMBER 16. AMBER ff14SB [23] force field was used for amino acids residues. The general Amber force field (GAFF) [24] was employed to describe the ligands. Sodium counter ions were also added near the most negative points of the prepared complexes to neutralise the systems [25]. The prepared complexes were immersed in TIP3P water box [26].

2.3.2. Minimization and MD simulation

Minimization of each complex started with steepest descent method (1000 steps). This was followed by 1000 steps of conjugate gradient method. Afterward, both minimized systems were hydrated in a 10 Å truncated box of TIP3P water model [26]. Both solvated complexes were minimized under the same conditions described above. An equilibration stage was performed for each system with SHAKE constraints on hydrogen atoms [27] with a cut-off distance of 12 Å. A time step of 2 fs was used in the simulation. In the production stage, each simulation was further performed to reach ten nanoseconds (ns) under constant pressure (NPT). The berendsen barostat method was used to control the pressure of both systems at 1 bar with an isotropic position scaling [28], the temperature was maintained at 310 K utilizing Langevin thermostat method [29].

2.3.3. MM-PBSA calculation

The MMPBSA.py modules utilized in AMBER 16, as well as, AmberTools 16 were used to calculate the free energy for both systems. The molecular-mechanical energy calculations were computed by Sander module within AMBER software. The overall free energy of binding was calculated via the following equations:

$$\Delta G_{\text{bind}} = \Delta H - T\Delta S \approx \Delta E_{\text{MM}} + \Delta G_{\text{sol}} - T\Delta S \quad (1)$$

$$\Delta E_{\text{MM}} = \Delta E_{\text{internal}} + \Delta E_{\text{electrostatic}} + \Delta E_{\text{vdw}} \quad (2)$$

$$\Delta G_{\text{sol}} = \Delta G_{\text{GB}} + \Delta G_{\text{SA}} \quad (3)$$

where, S is the entropy, ΔH is the enthalpy and T is the temperature in Kelvin. ΔE_{MM} describes the molecular mechanical (MM) energy change in the gas phase while ΔE_{int} is the internal energy, ΔE_{elec} is the coulomb electrostatic term and ΔE_{vdw} is the van der Waals interaction term. ΔG_{solv} is the solvation free energy, ΔG_{GB} is the electrostatic solvation energy (polar contribution) calculated by GB model and ΔG_{SA} is the nonelectrostatic solvation component (nonpolar contribution). The interval step of 10 ps for MM-GBSA calculation, and the salt concentration of 150 mM were applied. The residues around 5 Å from the ligand (PGE_2/inhibitor 4) were selected and their pairwise decomposition energies were computed using MM-GBSA module.

3. Results & discussion

In order to study the conformational changes upon ligand binding and identify the important residues that catalyze the oxidation reaction of prostaglandins, twenty nanoseconds (ns) molecular dynamics simulations were performed for two different systems of 15-hydroxyprostaglandin dehydrogenase.

3.1. Molecular docking

In this study, the crystal structure available in protein data bank contains only NAD^+ cofactor without a substrate or an inhibitor in the active site. Therefore, molecular docking studies were performed for PGE_2 and inhibitor 4 at the active site