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Molecular dynamics simulation of polmitoyl–coa hydrolase interactions with fmn, clofibrate, 2,4- dichlorophenoxy acetic acid ligands

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ABSTRACT

In enzymology, the palmitoyl-CoA hydrolase is the enzyme catalyzing the hydrolysis of acyl-CoA esters to free fatty acid and coenzyme A. This enzyme regulates the intracellular concentrations of acyl-CoAs, free fatty acids and CoASH. This enzyme show broad specificity towards its substrates, i.e. fatty acyl-CoAs with different chain length. Using different previously described inhibitors of palmitoyl-CoA hydrolase including Niacin, FMN, Ibuprofen, Dichlorophenoxy acetic acid (DCFA) and Clofibrate in molecular dynamic experiments we simulated enzyme complexes with its inhibitors separately. The simulation media were aqueous solution, 37°C and 1 atmosphere of pressure. In order to construct reasonable complexes between enzyme and its inhibitors we made molecular docking using HEX and Argus Lab software. Our results indicate that the hypothesized mechanism responsible for enzyme inhibition is the structural changes in enzyme protein induced by inhibitors that leads to increased content of irregular random structures in enzyme secondary structure. The structural alteration in enzyme is not necessarily manifested as protein denaturation but instead it appears as increase in protein compactness that brings the protein to go far from its native relaxed structure with enough flexibility and minimum tension. Our results also show that the more effective inhibitor induce the more compactness in protein structure in concomitant with increase in random structures.

Introduction

Human palmitoyl-CoA hydrolase or palmitoyl-CoA thioesterase (EC: 3.1.2.2) the enzyme catalyzing the following reaction:

$$Palmitoyl-CoA + H_2O \rightarrow Palmitate + CoASH$$

The enzyme has 35KDa of molecular weight and uses a magnesium ion and pH 6.5 for optimum action. The enzyme is predominantly found in cytosol, mitochondria and peroxisome. The enzyme belongs to the family of hydrolases which acting on thioester bonds. Other names of this enzyme are long chain fatty acid hydrolase, palmitoyl CoA thioesterase and palmitoyl CoA deacylase [1-2].

palmitoyl-CoA hydrolase has an important role in lipid metabolism, i.e. having role in fatty acid esterification or de esterification, controlling cellular beta oxidation, fatty acid traffic through membrane, signal transduction and even in gene regulation. Defects in palmitoyl-CoA hydrolase leads to decrease in keton body production, hypoglycemia and dysfunction of fatty acid dependent tissues. Enzyme inhibition by inhibitors, e.g. ibuprofen, valproate and 2, 4-dichlorophenoxyacetate increases mitochondrial membrane permeability leading to uncoupling of oxidative phosphorylation. In physiological condition, fasting conditions significantly decreases the mitochondrial activity of palmitoyl-CoA hydrolase and hence causes elevated levels of intra mitochondrial long-chain acyl-CoA accumulation [3].

It have been shown that administration of the antilipemic drugs such as clofibrate causes significant reduction in body weight via increasing cellular beta-oxidation and mitochondrial uncoupling. The plasma concentrations of cholesterol and triglyceride decreased in treated animals with antilipemic drugs [4]. The inhibition of palmitoyl CoA hydrolase by antilipemic drugs are reversible and the drugs lowered the palmitoyl CoA-binding capacity of microsomes [5].

Molecular dynamic (MD) is a powerful technique used to simulate protein-protein, protein-nucleic acid and protein-ligand complexes. Basically, MD simulations gives a valuable and reliable information about enzyme-inhibitor interactions. Based on this fact we used MD method to simulate our enzyme-inhibitor systems in hope of understanding the nature of interactions and the mechanisms by which inhibitors inactivate palmitoyl-CoA hydrolase activity [6-9].

Materials and methods

Crystal structure of human palmitoyl-CoA hydrolase, PDB ID: 2QQ2, with resolution of 2.8 A˚ and R-value of 0.265 obtained from the protein data bank, http:// www.rcsb.org/pdb. Coordinate files for inhibitors were constructed using ArgusLab 4.0.1 sotware [10] and energy minimized prior to docking experiments. Docking of inhibitors to palmitoyl-CoA hydrolase was carried out using Hex software version 5.1 (http://www.loria.fr/~ritchied/hex/). Hex is an interactive program for flexible docking of protein-protein and small ligands to protein either to fixed binding sites or to whole the protein structure [11-13]. Docking results were scored according to the complex binding energy and the best solutions of docking have chosen and used for further studies. Inhibitors topologies

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and coordinates in GROMACS format were obtained using The Dundee PRODRG2 Server [13]. Each simulation initiated with 1500 steps steepest decent minimization followed by 100 ps of system equilibration. A total 2 nanoseconds and for more confirmation 10 nanoseconds simulation was carried out for each of palmitoyl-CoA hydrolase-inhibitors complexes described above at 310 K and 1 atmosphere of pressure. The time steps of 1 femtosecond applied to all simulations. Molecular Dynamics (MD) simulations performed using double-precision MPI version of GROMACS 3.3.1 installed on UBUNTU version 9.10. Each structure of palmitoyl-CoA hydrolase-inhibitors was placed in the center of a cubic box with dimensions of 5.155 ×6.067×6.189 nm and filled with SPCE water molecules [14-15]. LINCS algorithm was used to apply constrain on bonds lengths. The SETTLE algorithm used to constrain the geometry of water molecules. In the MD protocol, all hydrogen atoms, ions, and water molecules were first subjected to 1500 steps of energy minimization by steepest descent and the energy of system were minimized to at least 300kJ/mol. The systems then submitted to a short molecular dynamic with position restrains for period of 100 ps and afterwards performed a full molecular dynamics without restrains [16].

Conflicts of Interest: There are no conflicts of interest to disclose.

Results and Discussion:

In order to show the reliability of MD experiments results obtained from our simulation we followed up temperature changes of the system during simulation figure 1. The constancy of temperature at 310K indicates the stability of the system. Changes in total and kinetic energy also confirmed the satiability of our system. In this context, the ratio of Kinetic/Total energy is a bit better index for the prevalence of stability in system. This ratio in our system is completely constant and it did not exceed 0.05 which in turn show the conservation of energy during simulation (data not shown)

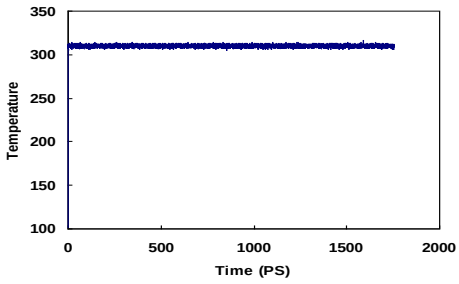

Figure 1: changes in system temperature during simulation around 310K. The data were obtained in aqueous solution, 310K and 1 atmosphere of pressure.

Applying MD run on enzyme-inhibitor complex at 310K temperature and 1 atmosphere of pressure in aqueous solution should affects their interaction and should exert structural alteration in enzyme structure. Secondary and tertiary structure analysis reveals that inhibitors are acting effectively on protein secondary structure. Figure 2 show the changes in random coil of palmitoyl-CoA hydrolase in the presence of different inhibitors. From inhibitors used in this study Niacin, the more effective inhibitor and FMN the less effective inhibitor show the most and the least increase in random coil respectively. Disturbing secondary structure seems to be the first probable mechanism by which inhibitors may inactivate palmitoyl-CoA hydrolase activities.

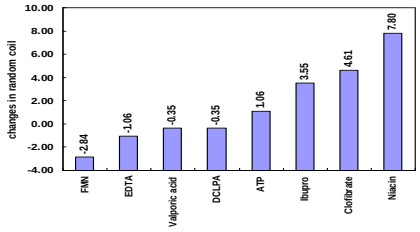

Figure 2: changes in random coil structure of palmitoyl-CoA hydrolase in the presence of different inhibitors. The data were obtained in aqueous solution, 310K and 1 atmosphere of pressure.

From misalliances data obtained from trajectory analysis that could be easily interpreted are surface accessible area, gyration radius and RMSD changes of protein during simulation. The first parameter, the accessible surface area (ASA) of protein, is the surface area in $Å^2$ of palmitoyl-CoA hydrolase protein that is accessible to a non-aqueous solvent [17]. Any change in ASA during simulation indicates structural changes in tertiary structure.

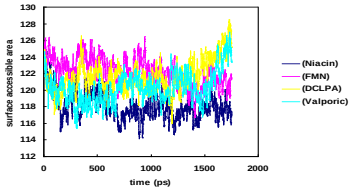

Figure 3: the surface accessible area of palmitoyl-CoA hydrolase in the presence of different inhibitors. The data were obtained in aqueous solution, 310K and 1 atmosphere of pressure

Figure 3 show that Niacin in contrast to FMN more effectively decreases surface accessible area of protein. This finding indicates that Niacin probably acts via increaseing protein compactness that in turn leads to decrease in disturbance of protein structure and necessarily induces increase in random coil proportion. The next parameter we studied was the radius of gyration of palmitoyl-CoA hydrolase or *Rg*. Radius of gyration is the root mean square distance of protein atoms from protein centroid. *Rg* describes the overall spread of the molecule and determines the protein structure compactness, the more *Rg* the less compactness of protein structure [18]. Figure 4 show the *Rg* change during simulation in the presence of different inhibitors.

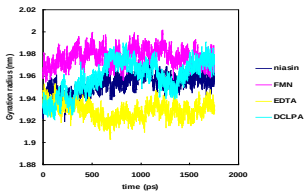

Figure 4: gyration radius of palmitoyl-CoA hydrolase in the presence of different inhibitors. The data were obtained in aqueous solution, 310K and 1 atmosphere of pressure.

As it is evident Niacin in comparison to FMN decreases the enzyme radius during simulation which is in confirmatory with surface accessible area changes. Figure 5 show the Root Mean Square Displacement (RMSD) of palmitoyl-CoA hydrolase - Inhibitor complexes. RMSD changes in Figure 5 show: first, the palmitoyl-CoA hydrolase -inhibitors complex reaches the equilibrium state over 1ns of simulation. Second the more increase in RMSD for inhibitors means the more interaction of

inhibitors with palmitoyl-CoA hydrolase during simulation as it seen for Niacin and this is reflected in more changes in palmitoyl-CoA hydrolase to inhibitors distance. Third less increase in RMSD as in FMN and EDTA reflect the less effect on enzyme structure and the less inhibitory potency.

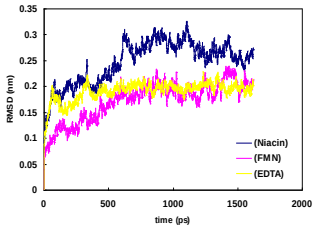

Figure 5: Root Mean Square Displacement (RMSD) changes of palmitoyl-CoA in the presence of different inhibitors. The data were obtained in aqueous solution, 310K and 1 atmosphere of pressure.

Conclusion:

Overally in case of palmitoyl-CoA we can conclude that enzyme inhibitors bind to enzyme, increasing its compactness, increasing its random structures and its surface accessible area and decreasing its gyration radius convert the native, relaxed and functional structure of enzyme to deformed, tens and non-functional structure without catalytic activity. The more effective inhibitor make the more potent inhibitor for palmitoyl-CoA enzyme.

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