Journal of Advanced Bioinformatics Applications and Research
ISSN 0976-2604.Online ISSN 2278 – 6007
Vol 4, Issue3, 2013, pp60-66
http://www.bipublication.com

IN SILICO ASSESSMENT OF *PPARγ* VAL 290 MET MUTANT STRUCTURE AND MOLECULAR DOCKING WITH THIAZOLIDINEDIONE OF TYPE-2 DIABETES

Ch. Kalyan Kumar* and Kaiser Jamil

Jawaharlal Nehru Institute of Advanced Studies (JNIAS)
Buddha Bhavan, M.G Road, Secunderabad - 500 003 Andhra Pradesh, India
Corresponding author: E-mail: biotech.kalyan@gmail.com, Tel: 040-27541551, Fax: 040-27541551

[Received-24/09/2012, Accepted-10/10/2013]

ABSTRACT:

Peroxisome proliferator-activated receptor (PPAR) which is a transcription factor from super family of nuclear receptors regulates the expression of genes. PPAR-g has strong effects on various diseases including carcinogenesis & type-2 diabetes. The present study was aimed at predicting and understanding PPAR Gamma structure and function by creating a homology model using Schrodinger 2009. The proteins were minimized for 20,000 steps. The potential energy of wild protein was -1.1450679e+06 and the potential energy of mutant protein was -1.1597088e+06. Docking of thiazolidinedione ligand to the wild and mutant proteins was showed hydrogen bonds with Tyr 473 and Ser 289. The docking score was -3.619480. These hydrogen bonds were lost when the ligand is docked to the mutant protein. The ligand did not form any hydrogen bonds with the mutant protein. The docking score was -2.723570. The protein with less energy will be more stable. Therefore, the increase of energy in the mutant protein shows that it may be less stable than the wild protein. The docking results show that when Val 290 Met mutation occurs, the ligand does not bind properly to the protein. This study revealed that the binding energies for mutated PPAR Gamma structures of the proteins were greater than that of the wild type proteins. This indicates that mutations causing structural modifications modulated the drug binding energies with various ligands (drugs). It therefore shows that the variations in the structure of the proteins influences the drug-binding capacity and also influences drug toxicity related to drug-gene interactions.

Key Words: PPAR (Peroxisome proliferator-activated receptor) BLAST (Basic Local Alignment Search Tool), PDB (Protein Data Bank).

[1] INTRODUCTION

Type 2 diabetes (T2D) poses a major health problem globally, especially in many developing countries [1]. It is brought about when the cells in the muscles, liver, and fat tissues fail to utilize insulin effectively. Human body has to maintain the blood glucose level at a very narrow range, which is done with insulin and glucagon [2]. The function of glucagon is causing the liver to release glucose from its cells into the blood, for the production of energy. The worldwide

prevalence of diabetes for all age groups was estimated to be 2.8% in 2000 and it is projected to be 5.4% in 2025 [3]. Although there are growing numbers of type 2 diabetic patients, the available remedy for this disease is confined to a limited number of drug classes. The most frequently prescribed medication for the treatment of type 2 diabetes is the group of thiazolidinediones [4]. This class of drugs acts through the nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ), and its activation can be exploited to improve insulin sensitivity in insulin-resistant animals or humans [5**]** However, the mechanism by which and the tissues in which TZDs act to increase insulin sensitivity remain unclear [6,7]

Peroxisome proliferator-activated receptors (PPARs) belong to the nuclear receptor super family of ligand inducible transcription factors that were identified in the 1990s in rodents, named after their ability to induce the proliferation of peroxisomes [8]. PPARγ was identified as the master regulator of adipogenesis and recognized to play a key role in glucose and lipid metabolism and thereby affecting insulin sensitivity, it was obvious to search for PPARγ mutations in diabetic patients. Yen et al. were the first who performed a molecular scanning of the human PPARG gene in diabetic Caucasians with or without obesity [9]. Several mutations in the PPAR-γ gene have been detected in human subjects. A $Pro^{12} \rightarrow$ Ala mutation that reduces the transcriptional activity of PPAR-γ is associated with lower BMI and improved insulin sensitivity [10, 11]. Furthermore, two dominant negative mutations of PPAR-γ, $Pro^{467} \rightarrow$ Leu and $Val^{290} \rightarrow$ Met have been identified in individuals with severe insulin resistance [12]. These genetic studies suggest that PPAR-γ also contributes to lipid and glucose metabolism in the body as a whole. In this work, the first three-dimensional molecular model of the hypothetical structures for the wild-type and mutants of PPARγ $Val^{290} \rightarrow$ Met mutation were elucidated by a homology modeling method. In addition, the orientations and binding affinities with those new structures were investigated. Hence, in this study we attempt to utilize this empirical structural data to analyze to the impact of $Val^{290} \rightarrow$ Met mutation on TZDs resistance.

[2] MATERIALS AND METHODS

Computational methods

The methodology was described in four steps: Identifying a suitable template, making an optimal target template alignment, building the model and validating the model. Protein structure Prediction and homology modeling has recently been reviewed Different online tools were used to select the protein of interest (PPAR Gamma-2) and determine structures as the target 3D structure.

Protein databank

The RCSB PDB provides a variety of tools and resources for studying the structures of biological macromolecules and their relationships to sequence, function, and disease. The RCSB is a member of the PDB whose mission is to ensure that the PDB archive remains an international resource with uniform data. This site offers tools for browsing, searching, and reporting that utilize the data resulting from ongoing efforts to create a more consistent and comprehensive archive.

Identifying suitable template and sequence alignment

Heuristic search methods such as BLAST [13] and FASTA [14] were used to find initial template (PPAR Gamma-2 protein). The program compares nucleotide or protein sequence databases and calculates the statistical significances of matches. Blast can be used to infer functionally and evolutionary relationships between sequences as well as help identify members of gene families. Our query sequence was blast against PDB database using blast in NCBI blast, using default parameters except the

matrices, and results obtained were reported,. The chosen templates were the sequence from the latest version of the PDB databank with the lowest expected value and highest score after four iterations. Alignment of sequences with their templates structure was done using the alignment command in Schrödinger tool. The software also into account structural information from the template when constructing an alignment. The Schrödinger was used for aligning all target sequence with their corresponding template sequence in the PDB file.

Homology modeling using Schrödinger tool

The Homology Modeling workflow incorporates the complete protein structure prediction process from template identification, to alignment, to model building. Refinement can then be done from a separate panel, and involves side-chain prediction, loop prediction, and minimization. The Prime suite provides the tools for four main tasks: homology modeling, structure refinement, covalent docking, and binding energy estimation, all of which are accessible from the Maestro interface. Homology modeling can be done through one of two interfaces, the Structure Prediction wizard, and the Multiple Sequence Viewer. In both you can perform the required steps leading from a protein sequence to the construction of a 3D structure.

Model Building

To generate the core of the model, the backbone atom positions of the template structure are averaged. The templates are thereby weighted by their sequence similarity to the target sequence, while significantly deviating atom positions are excluded. The template coordinates cannot be used to model regions of insertions or deletions in the target-template alignment. To generate those parts, an ensemble of fragments compatible with the neighboring stems. The best loop is selected using a scoring scheme, which accounts for force field energy, steric hindrance and favorable interactions like hydrogen bond formation. If no suitable loop can be identified, the flanking residues are included to the rebuilt fragment to allow for more flexibility.

Structural Visualization & Energy Minimization.

For structure visualization and energy minimization gromacs was used. The structure was imported in Gromacs 4.5.5 software powerful, unified multiplatform graphical user interface. Based on this structure, a structure was built with three template structures using Gromacs and was saved as PDB structures, which had comparatively lower energy and required less energy minimization iterations.

Docking With Drugs

TZDs were docked into the structures of wild type and mutant type using Glide module of Schrodinger. In this docking simulation, semi flexible docking protocols [15] were used, in which the protein structures were kept rigid and the TZD being docked was kept flexible. The best docked complexes based on the lowest binding energy were further analyzed for hydrogen bonding interactions and the binding energy of wild type and mutant type was compared.

[3] RESULTS

In the present study, 3D homology modeling was carried out for the Peroxisome proliferator-activated receptor (PPAR). The primary 3D structure of the peroxisome proliferator-activated receptor (PPAR) human protein sequence was selected from the NCBI and loaded as the raw sequence. Schrödinger software was used to generate homology models. The threading of target PPAR gamma sequence with template sequence was performed. The sequence with low E values was selected from different homologous gene sequences. The protein model built in this study was ribbon shaped which retained the general helical structure [Figure-1]. At this point

we have a template structure and a good sequence alignment. The next step is to automatically build a series of homology models and select the highest quality one. For this step we will use the Build Homology Models .To increase the quality of the homology models built, especially for homology models of protein, it is very important to determine which cystines are disulfide-bonded and specify these in the Build Homology Models protocol. When building homology models, it is often recommended to build several models in order to have a larger pool from which to select a high quality model [Figure-2]. PROCHECK analysis revealed the residues within the limits of Ramachandran plot. The Ramachandran plot also showed 93.5% of residues in most favored regions with no residues in disallowed region.

The model has to be optimized because the changed side-chains can affect the backbone, and a changed backbone will have effect on the predicted rotamers. Optimization can be done by performing refinements using Molecular Dynamics simulations of the model. The model is placed in a force-field and the movements of the molecules are followed in time, this mimics the folding of the protein. The big errors like bumps will be removed but new smaller errors can be introduced. The calculated energy should be as low as possible. The proteins of PPAR gamma were minimized for 20,000 steps. The PPAR gamma2 protein was taken (PDB ID: 3PRG) and prepared using the protein preparation wizard of Schrodinger 2009. The Val 290 amino acid is mutated to Met using the Mutate residue option present in Maestro. These wild and mutant proteins were used for Energy minimization and docking studies. Energy Minimization of the wild and mutant proteins was carried out using Gromacs 4.5.5 software with gromos53 force field. The proteins were minimized for 20,000 steps. The potential energy of wild protein was -1.1450679e+06 and the potential energy of mutant protein was -1.1597088e+06. The stability of a protein depends on its energy. The protein with less energy will be more stable. Therefore, the increase of energy in the mutant protein shows that it may be less stable than the wild protein. Docking of thiazolidinedione ligand to the wild and mutant proteins was carried out using the Glide module of Schrodinger. XP docking was done in both the cases. Docking of the ligand with wild protein showed hydrogen bonds with Tyr 473 and Ser 289. The docking score was -3.619480. These hydrogen bonds were lost when the ligand is docked to the mutant protein. The ligand did not form any hydrogen bonds with the mutant protein. The docking score was -2.723570. The docking results show that when Val 290 Met mutation occurs, the ligand does not bind properly to the protein [Figure-3].

[4] DISCUSSION

The three-dimensional structure is vital in understanding how proteins function and this understanding is a crucial step in designing and engineering new proteins with specific functions. The unique sequence of a protein is so important for function, that the mutation of any single amino acid can disrupt the entire fold and thereby function of a protein by upsetting the balance of interacting forces within the protein. Structure based calculations of the relative activity of a protein after a mutation is an important problem since small mutations can lead to complete loss of function of the protein, and subsequently, to disease. However, predicting the functional outcome of mutations is very difficult, even when sufficient structural data are available. To date, few studies exist that have tried to correlate mutant protein function with biochemical measurement, and in fact, those that have been successful examine the stability of the protein, not the functional outcome. In the present study, the studied protein has been chosen for the fact that there is much information known about these proteins that can be used to gain even more

knowledge. Even though extensive research has been performed in connection to these proteins, there are also many unanswered questions left. Some have structural information missing, some miss known connections between function and disease and some lack information regarding protein interactions. The protein studies done in this project try to fill some of these gaps in knowledge.

The protein PPAR Gamma is studied from a mutational perspective, where we examine how the mutations affect the stability and function of the protein. Previous studies suggest that this mutation alters the ability of PPAR-gamma regulate the expression of metabolically important genes. PPAR Gamma has strong effect on various diseases including type-2 diabetes. The homology modeling workflow incorporates the complete protein structure prediction process is done by template identification, to alignment, and model building. The template for PPAR gamma (peroxisome proliferated activated receptor gamma) is identified from the PDB (Protein Data Bank) file which is 3PRG ,and the sequence of protein is found through ,NCBI (National Center for Biotechnology Information) with the help of BLAST. By which a suitable homologous protein of which the structure has been solved. The first identified loss-of-function mutations Val290Met (also called V318M), and Pro467Leu (also called P495L). These Val290Met and Pro467Leu mutations drastically reduced transcriptional activity of PPAR-γ in a dominant-negative fashion in vitro and resulted in a severely insulin resistant though lean phenotype in vivo. Structure-function simulations suggested that these mutations affect the orientation of helix 12 of PPAR-γ, which is important for the interaction with ligands and co activators. In the present modeling study, we found that the potential energy of wild protein was -1.1450679e+06 and the potential energy of mutant protein was -1.1597088e+06. The stability of a protein depends on its energy. The protein with less energy will be more stable. Therefore, the increase of energy in the mutant protein shows that it may be less stable than the wild protein. Our results found that amino acid substitution in PPAR gamma binding sites upon ligand binding to the binding site shape, which, in turn, limits ligand entry.

Hence, studies using the docking program could determine the best fit between the ligand and the target, the binding site can be seen in a variety of possible ways. In other words, we are using it as a conformation generator for conformations that fit into the active site. The docking program allows us to focus on a set of conformations for each molecule that fit into the binding site. Additionally, the docking program performs the alignment of all the conformations to one another, which is much simpler than dealing with the combinatorial explosion of ways in which the active compounds can be aligned and the number of features which are required. According to previous works, one missense mutation is sufficient to lead to altered binding pattern of drug targets. Further we studied docking of thiazolidinedione ligand to the wild and a mutant protein was carried out using the Glide module of Schrodinger. XP docking was done in both the cases. The docking results show that when Val 290 Met mutation occurs, the ligand does not bind properly to the protein. Our hypothesis brings some light to the understanding of the molecular basis of PPAR selective ligands mode of interaction and may be helpful in further rational design of PPAR selective agonists. This is the first computational report for these drug gene interactions, which opens up the avenues for personalized medicine, since the differences in structure (due to mutations or SNPs) of the targets influences the dose response of the therapeutic agents.

[5] CONCLUSION

This study revealed that the binding energies for mutated PPAR Gamma structures of the proteins were greater than that of the wild type proteins. This indicates that mutations causing structural modifications modulated the drug binding energies with various ligands (drugs). It therefore shows that the variations in the structure of the proteins influences the drug-binding capacity and also influences drug toxicity related to drug-gene interactions. This is the first computational report for PPAR Gamma-2 gene interactions in type-2 diabetes. This could be a model study for drug designing or selecting a drug suitable to the individual patient's genomic response.

FIGURES

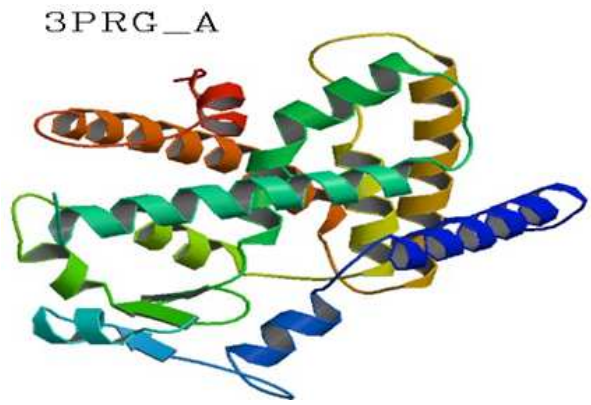

Fig: 1 3PRGA from PDB file

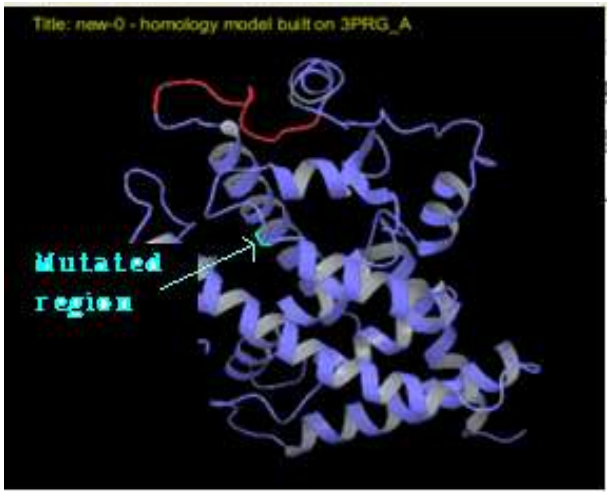

Fig: 2 Homology model of PPAR gamma built on 3PRG_A

BLAST search for template: 3PRG_A is selected for Blast with our Query sequence.

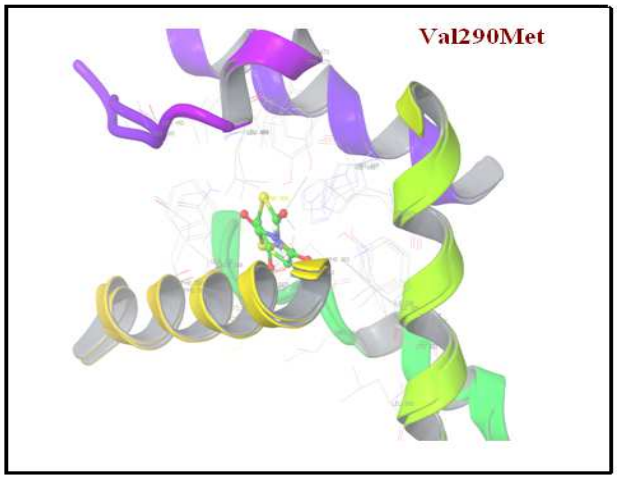

Fig:3 The structural alignment of Protein-Ligand complexes of wild and mutant proteins shows that the ligand interacts with the wild type protein whereas these interactions are lost in the mutant protein.

REFERENCES

1. Iwanishi, M. and Kobayashi, M, (1993), Effect of pioglitazone on insulin receptors of skeletal muscles from high-fat-fed rats, Metabolism. 42, 1017-21.
2. Zimmet, P.K.G, Alberti, K.G, Shaw. J, (2001), Global and societal implications of the diabetes epidemic, Nature. 41, 782–7.
3. Cho, H.J, Park, J, Lee, H.W, Lee, Y. Kim, J.B, (2004), Regulation of adipocyte differentiation and insulin action with rapamycin, Biochem. Biophy. Res. Commun, 21, 942-948.
4. Patel, C. B, Delemos J.A, Wyne, K.L, McGuire, D.K, (2006), Thiazolidinediones and risk for atherosclerosis: pleiotropic effects of PPar gamma agonism, Diab Vasc Dis Res. 2, 65–71.
5. Saltiel, A.R, Olefsky, J.M, (1996), Thiazolidinediones in the treatment of insulin resistance and type II diabetes, Diabetes. 45, 1661–1669.
6. Olefsky, J.M, (2000) Treatment of insulin resistance with peroxisome proliferator-activated receptor γ agonists. *J Clin Invest* **106**:467–472
7. Kersten, S. Desvergne, B. Wahli, W, (2000), Roles of PPARs in health and disease, Nature. **405**, 421–424.

8. Kim, J.B, Wright, H.M, Wright, M, Spiegelman, B.M, (1998) ADD1/SREBP1 activates PPARgamma through the production of endogenous ligand, Proc Natl Acad Sci U S A. 95, 4333-4337.
9. Garofalo, R.S, Orena, S.J, Rafidi, K, Torchia, A.J, Stock, J.L, Hildebrandt, A.L, Coskran, T, Black, S.C, Brees, D.J, Wicks, J.R, McNeish, J.D, Coleman, K.G, (2003) Severe diabetes, age-dependent loss of adipose tissue, and mild growth deficiency in mice lacking Akt2/PKB beta, J Clin Invest. 112, 197-208.
10. Deeb, S.S, Fajas, L, Nemoto, M, Pihlajamäki, J, Mykkänen, L, Kuusisto, J, Laakso, M, Fujimoto, W, Auwerx, J, (1998), A Pro12Ala substitution in PPARγ2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity,. Nat Genet. **20**, 284–287.
11. Masugi, J, Tamori, Y, Mori, H, Koike, H, Kasuga, M (2000), Inhibitory effect of a proline-to-alanine substitution at codon 12 of peroxisome proliferator-activated receptor-γ2 on thiazolidinedione-induced adipogenesis, Biochem Biophys Res Commun. 268, 178–182.
12. Barroso, I, Gurnel,l M, Crowley, V.E.F, Agostini, M, Schwabe, J.W, Soos, M.A, Maslen, G.L, Williams T.D.L, Lewis, H, Schafer, A.J, Chatterje,e V.K.K, Rahilly, S, (1999), Dominant negative mutations in human PPARγ associated with severe insulin resistance, diabetes mellitus and hypertension, Nature, 402, 880–883.
13. Altschul, S. (1991), Amino acid substitution matrices from an information theoretic perspective, J. Mol. Biol. 555–565.
14. Pearson, W. (1998) Empirical statistical estimates for sequence similarity searches, J. Mol. Biol. 276, 71–84.
15. Morris, G.M, Goodsell, D.S, Halliday, R.S, Huey, R, Hart, W.E, (1998), Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function, J Comput Chem. 19, 1639-1662.