



An In silico approach to design the mutated 3D structure of BCHE using AB initio method and testing the efficacy of the selected ligands in inhibiting the S226G mutation

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Abstract:

Butyryl cholinesterase (BCHE) is a protein belonging to ester lipase super family. It hydrolyzes choline esters in addition to its role in nerve impulse transmission. BCHE has three active sites located at 226, 353 and 456 which are responsible for acyl ester intermediate, and charge relay system. Among the three sites, 226 (serine) have been reported to have the natural variant S 226 G. Alteration at this site affects the functionality of the protein due to its importance in the active site formation. Variations in this gene have also been reported in BCHE deficient conditions. BCHE is implicated to have a role in lipid metabolism; any alteration to its function causes a hindrance to the mobilization of fats leading to obesity which causes Type II Diabetes. Present work involves complete analysis of BCHE gene with reference to the site S226G and designing the mutated BCHE structure using AB Initio approach. Docking studies were performed with potent lead molecules with an aim to inhibit or minimize the adverse effect of the mutation.

Keywords: BCHE, Docking, Mutation, Obesity, Lipid Metabolism, Type II Diabetes.

Introduction:

Diabetes has become one of the most common diseases among the people of all age groups. Type 2 diabetes also known as “Non insulin dependent diabetes mellitus” is characterized by a defect in the insulin activity. It is caused due to the impairment in the insulin signaling mechanism, as a consequence of insulin resistance. Large number of proteins has been reported to be involved in the onset of diabetes; BCHE is one such protein, whose exact role on the onset of the condition is still an enigma. Cholinesterases are a class of proteins

involved in the hydrolysis of cholines. The two cholinesterases, ACHE and BCHE though belong to the same class they differ in their substrate requirement. ACHE is specific to acetylcholine as its substrate, whereas BCHE is called non-specific esterase or pseudo cholinesterase as it do not show substrate specificity, it hydrolyses wide range of esters, especially the depolarizing neuromuscular blocking agent succinylcholine. ACHE and BCHE both belong to a super family “Ester Lipases” [1] which indicates that apart from their esterase activity they are also involved in lipid metabolism. This can be considered for their role in Obesity.

BCHE is a protein of 602 amino acids in length located on the q arm of the 3rd chromosome. It belongs to the family of alpha beta hydrolases [2] based upon the structural motifs present in it. It is known to play a vital role in Diabetes, Adiposity and Nerve disorders [3]. In vertebrates, it appears in the serum, hemopoietic cells, liver, lung and heart, at cholinergic synapses and in the central nervous system [4]. The mutations in BCHE are known to have various effects in the normal metabolism of the body. BCHE is reported to have a role in insulin resistance [5], one of the causes for the onset of Diabetes. It also has a role in most of the neurodegenerative disorders like Alzheimer's disease, Parkinson's disease etc.

Drug designing enables to search the possible orientations as well as conformations of the ligand, binding to the target protein and evaluating the generated binding mode of the drug candidates. Drug discovery and development is an intense, lengthy and an interdisciplinary endeavor. It majorly involves three steps Designing the Receptor, Designing the Target and Performing the Docking. The target defines the protein receptor that receives the drug and the Drug acts as a ligand and binds to the specific sites on the protein receptor called the binding sites. The quality of the

docking is assessed based on the binding properties and the energy values of the complex after docking, which are the indicators of stability.

Materials and Methods:

Protein analysis using BioAxcell:

BioAxcell, a commercial Bioinformatics package was used to perform the initial protein analysis which includes the *Multiple Sequence Alignment* and domain analysis. The Package provides a direct and a most user friendly access to several other analysis tools which has not been used in the current study.

Structural analysis:

Various structure analysis tools from EXPASY [6] proteomic server were used to analyze the secondary and tertiary structure of BCHE protein.

Ab-initio Approach:

The structure of the mutated protein has been modeled using Ab-initio approach, which predicts the structure for the given sequence based on sequence similarity, it employs threading approach.

3D Structure Prediction:

I-TASSER server [7] had been used to predict the structure of mutated protein based on ab-initio approach. Process was completed with four sequential steps: template selection, target-template alignment, model construction and model refinement and assessment.

Template Sequence Search

The template sequence having high degree of sequence similarity to the target protein is selected based on PDB BLAST.

Model Assessment:

The generated model is tested with different methods to find the errors, disorder regions, quality of the generated model etc. The process also deals with the model verification, calculation of energies, calculation of main bond length, main bond angles etc. PROCHECK SERVER and VERIFY 3D are used for this purpose.

The PROCHEK server [8] is an analysis tool that provides an idea of the stereo chemical quality of all protein chains of a PDB structure. The VERIFY-3D is a structure evaluation server that analyzes the compatibility of an atomic model (3D) with its own amino acid sequence

Disorder prediction:

To identify the mutational hotspots that may be possible in the protein sequence DISEMBL [9] is used. The presence of the target amino acid in any of these mutational sites strengthens the importance of the mutation.

Active site and binding site prediction:

Q SITE FINDER, POCKET FINDER and CASTP were used to identify the potential active sites within the protein. These sites indicate the regions of high ligand binding affinity.

Generation of Lead library:

PUBCHEM and CHEBI data bases were employed to create the drug library of the lead molecules [10]. These databases provide the list of all the chemical compounds with their molecular properties which can be used as a drug candidate to mitigate the metabolic defect.

Screening of lead molecules:

Once the lead library is generated they must be subjected to prior screening to identify the potential compounds that has least ambiguity and can be used as a drug. Screening involves various stages for which MOBYLE ADME SERVER, DUNDEE PRODRG SERVER are employed [11, 12].

Energy Minimization:

The receptor and the ligand were subjected to energy minimization. AMBER and steepest descent method were used in tripose force field.

Docking:

Docking is frequently used to predict the binding orientations of drug candidates to protein targets in order to predict the affinity and activity of the small molecule. GOLD and MOLEGRO [13] were used for docking.

Results and discussion:

Sequence Alignment:

Alignment shows that both BCHE and ACHE share a considerable sequence similarity which was more than 50%, which can be considered as an indication for their structural similarity.

Domain Analysis:

Domain analysis indicates that BCHE has three overlapping domains, an Abhydrolase, an ACHE and a CO Esterase. The three proteins ACHE, BCHE and HSL share a common domain Abhydrolase which is an indication of their functional similarity.

Structure analysis:

Structure of BCHE [14] showing target amino acid SER (226)

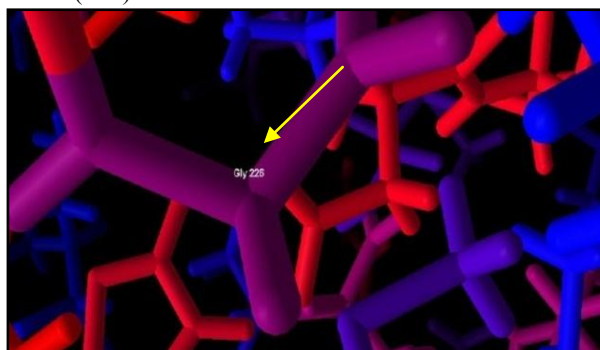


Fig1: The above figure shows the target site, Glycine 226.

Ab initio approach for model generation of the mutated protein:

Table: 1 Five models were generated through ab initio approach

Model no.	c score
1	-1.22
2	-1.46
3	-1.66
4	-1.47
5	-2.53

Table 1: C-score is a confidence score for estimating the quality of predicted models by I-TASSER.

Model Screening:

Models were validated using Ramachandran plot. From a total of five models, Model3 and Model5 were rejected due to the absence of glycine at the desired position (226). The other models were selected for further screening.

Energy Evaluation of Model 1, Model 2, and Model 4:

Molecular Dynamics study has been performed by applying AMBER force field and the models were subjected to energy minimization using Conjugate gradient method. From the results model 1 was selected as it was most energetically favorable compared to the other structures.

Table: 2 Model Evaluations

Model no.	c score
1	-1.22
2	-1.46
3	-1.66
4	-1.47
5	-2.53

Table 2: C-score is a confidence score for estimating the quality of predicted models by I-TASSER.

Model Screening:

Models were validated using Ramachandran plot. From a total of five models, Model3 and Model5 were rejected due to the absence of glycine at the desired position (226). The other models were selected for further screening.

Energy Evaluation of Model 1, Model 2, and Model 4:

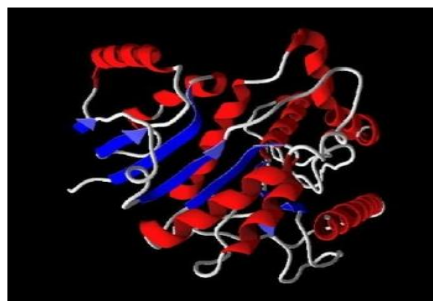
Molecular Dynamics study has been performed by applying AMBER force field and the models were subjected to energy minimization using Conjugate gradient method. From the results model 1 was selected as it was most energetically favorable compared to the other structures.

Table: 3 Model Evaluations

Models	Initial energy (kcal/mole)	Optimized energy(kcal/mole)
1	151679.160	-13481.026
2	219354.761	-13228.543
4	-569.479	-13113.982

Table 3: The above results show that Model 1 has lowest energy which makes it the most stable structure.

Fig: 2. Structure of Mutated protein designed by Ab initio method



Model Assessment:

The ERRAT results provided that the overall quality factor of the generated model is 76.599%. Q mean score showed that the quality of the model is 0.649 (ranging from 0 to 1). The results from DDFIRE/DFIRE2 showed that the total energy: -1357.27 which is equal to the summation of DFIRE2 energy and other three angle related energies. VERIFY 3D^[15] estimated that the atom no. 226 Glycine showed the average compatibility of 0.49 with 3D-1D profile score. The Ramachandran Plot obtained from the PROCHECK results gave the stereo chemical property of the model. It showed that the Residues in most favored regions were 92.0%, residues in allowed regions were 6.8%, and the residues in disallowed regions were 1.2%.

Ramchandran plot showing the quality of the generated model.

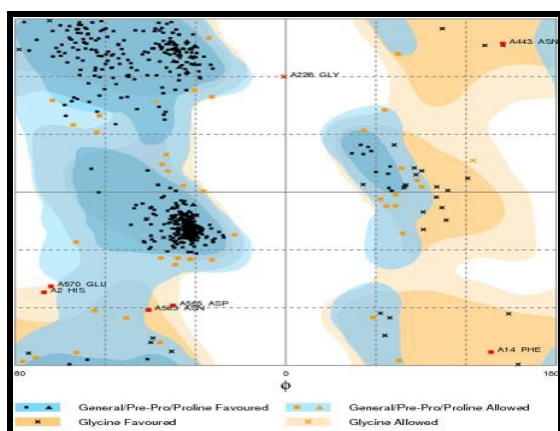


Fig 3: The above Ramchandran plot depicts that the generated model for mutated protein is acceptable.

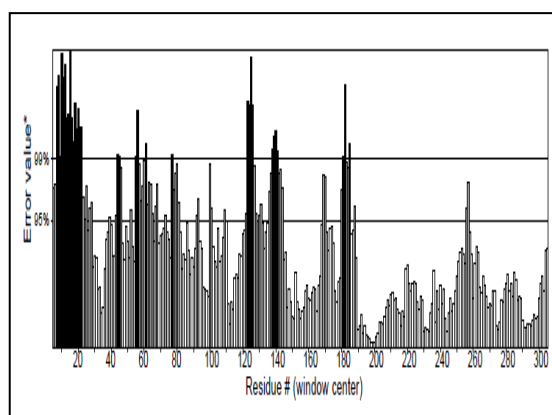
Plot obtained in ERRAT

Fig4: ERRAT showed the overall model quality to be 76.599%

Energy Evaluation study of the selected model:

Initial and Final optimized Energy details of the molecule.

Energy Parameters	Initial Energy	Final optimized Energy
Bond Stretching Energy	988.912	281.009
Angle Bending Energy	1363.749	1156.179
Torsional Energy	1567.680	1601.717
Improper Torsional Energy	61.967	65.940
1-4 van der Waals Energy	6952.321	2080.713
van der Waals Energy	145205.177	-4388.893
1-4 Electrostatic Energy	10936.835	5052.464
Electrostatic Energy	-15397.481	-19330.156
Total Energy	151679.160	-13481.026

Table4: shows the Mol dock score and gold fitness value of selected compounds. Compound with CID 137324 showed minimum mol dock score and maximum fitness value. This indicates that the compound can be selected as a potent chemical for the target site.

Disorder prediction:

The disorder prediction using DISEMBL showed that the target site 226 lies in the mutational hot spot.

Binding site prediction:

CASTP showed that the target 226 site was one of the good binding sites suitable for ligand docking. The pocket 113 contained the target site.

Selection of compounds:

To identify a potent chemical compound that can be used to target the mutated site of BCHE a LEAD LIBRARY was generated. 72 chemicals from PUBCHEM and CHEBI were screened at various levels. Finally 7 ligands were filtered which were further compared energetically to select the compound which is functionally and energetically favorable. The selected compound was N-Alanylproline-o-(4-nitrobenzoyl) hydroxylamine with CID 137324.

Docking results in Molegro and Gold

Ligand name	Mol Dock score	GOLD Fitness Value
1-(((2-((5-cyanopyridin-2-yl)amino)ethyl)amino)acetyl)-2-cyano-(s)-pyrrolidine	-123.74	31.66
N-alanylproline-o-(4-nitrobenzoyl)hydroxylamine	-130.35	41.50
Saxagliptin	-107.42	16.42
1-(((3-hydroxy-1-adamentyl)amino)acetyl)-2-cyanopyrrolidine	-109.60	23.89
Vildagliptin	-120.40	17.48
Diprotin A	-121.90	25.24
Topiramate	-118.846	38.07

Table 5: The above table shows the list of filtered compounds along with their Mol dock score and Gold fitness value.

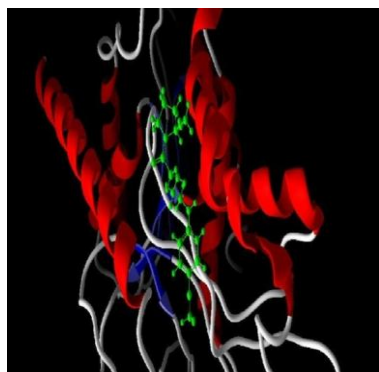
Docking of the target site with the ligand: CID 137324

Fig5: shows the docking of the target and the ligand (CID: 137324)

Discussion:

BCHE shows more than 50% similarity to ACHE which indicates their functional similarity (as indicated by EMBOSS analysis). They are mainly involved in Regulation of Nerve impulse transmission in addition to their role in lipid metabolism. BCHE is involved in the hydrolysis of succinyl choline thereby acting as a depolarizing agent highlighting its role in nerve impulse transmission [16]. The involvement of ACHE in lipid metabolism was considered due to its sequence similarity to HSL, a protein related to Acetyl cholinesterase and lipoprotein lipases

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.These class of proteins are involved in the mobilization of fats, thus playing a crucial role in the overall energy homeostasis in mammals [17]. Since ACHE is similar to both HSL and BCHE, the role of BCHE in lipid metabolism is interpreted. It can be hypothesized that a mutation to the BCHE gene and defect to its protein synthesis causes a hindrance for the fat mobilization, one of the well known causes of diabetes.

Due to the excessive accumulation of fat in the body, two major transcription factors FOXA2 and HNF1 are hampered. This in turn leads to the diminished function of Gnt -4 A glycosyltransferase which is required for the transportation of glucose and glucose sensing by the pancreatic beta cells [18].

The transporter defect will ultimately lead to glucose accumulation and diabetes mellitus type 2. The mutated structure has been designed using Ab initio threading approach to study the effect of the selected chemical compound on the protein. The ligands used for docking the targeted site of Bche are dipeptidyl peptidase inhibitors which can be used for the treatment of type 2 diabetes. Analysis showed that the region 118 to 251 of Bche protein has peptidase activity. It has been reported that using DPP4 inhibitors improves insulin sensitivity. As Bche plays its role in type 2 diabetes [19] via, insulin resistance, by using these drugs insulin sensitivity can be improved by blocking the effect of the mutated residue (226) thereby regulating insulin resistance and control of Type 2 Diabetes.

Conclusion:

From the above work it was studied that BCHE is one of the major protein that is involved in insulin resistance thereby being indispensable in diabetic study. One of the domains of BCHE is common to two other proteins, ACHE and HSL which implies their functional similarity reporting its role in lipid metabolism. S226G mutation of the protein is one of the mutational hot spot lying in the active site which has been considered as the target in this work .The compounds that can be used as suitable drug candidates have been selected and subjected for various screening procedures. The final ligands used for docking were 1-(((2-((5-cyanopyridin-2-

yl) amino) ethyl) amino) acetyl)-2-cyano-(s)-pyrrolidine, N-alanylproline-o-(4-nitrobenzoyl) hydroxylamine, Saxagliptin, 1-(((3-hydroxy-1-adamantyl) amino) acetyl)-2 cyanopyrrolidine, Vildagliptin, Diprotin and Topiramate. All the ligands were optimized using suitable software. Docking was performed in Molegro and GOLD softwares to select the best compound that can be used as a drug against BCHE. From the docking result analysis it was found that the compound named "N-alanylproline-o-(4-nitrobenzoyl) hydroxylamine is the best drug candidate due to its minimum Moldock score and maximum fitness value of it when compared to the other ligands.

To prove the efficiency of the selected ligand in treating the adverse symptoms of diabetes the work can be extended to clinical trials. This can be one of the approaches for the treatment of Diabetes.

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References:

- 1) Cygler et al "relationship between sequence conservation and 3 dimensional structures in a large family of esterases, lipases and related enzymes". Prot.sci .2.
- 2) Jerry Phelps "Misfolded protein presents potential molecular explanation for autism spectrum disorders". Environmental Health Perspectives.31 Jan, 2012.
- 3) Beben Benyamin et al. "GWAS of butyrylcholinesterase activity identifies Four novel loci, independent effects within BCHE and secondary associations with metabolic risk factors". Human molecular genetics, 2011.
- 4) Mack A, Robitzki A et al, "The key role of butyrylcholinesterase during neurogenesis and neural disorders: an antisense-5'butyrylcholinesterase-DNA study." PMID: 10739090.
- 5) Tomoyuki Iwasaki et al, "Serum butyrylcholinesterase is strongly associated with adiposity, the serum lipid profile and insulin resistance". Internal medicine. Vol 46(2007).

6) Gasteiger E. et al, "EXPASY: the proteomics server for in depth protein knowledge and analysis". Nucleic acids Res.31: (2003).

7) Yang Zhang "I-TASSER: Fully automated protein structure prediction in CASP8". Proteins: structure, Function and Bioinformatics, Vol. 77, No.S9. (21 August 2009).

8) R. A. Laskowski et al "PROCHECK: a program to check the stereochemical quality of protein structures" Journal of Applied Crystallography, Vol. 26, No. 2. (Apr 1993).

9) R. Linding et al "protein disorder prediction: implications for structural proteomics" Structure Vol 11, Issue 11, 4 November 2003.

10) Bolton E et al, "PubChem: Integrated Platform of Small Molecules and Biological Activities." Chapter 12 IN Annual Reports in Computational Chemistry, Volume 4, American Chemical Society, Washington, DC, 2008.

11) Miteva MA et al. "FAF-Drugs: free ADMA/tox filtering of compounds collections." Nucleic acids Res. 2006.

12) A. W. Schüttelkopf .et al "PRODRG - a tool for high-throughput crystallography of protein-ligand complexes." Acta Crystallogr. D60, 1355–1363. PMID15272157.

13) René Thomsen et al "MolDock: A New Technique for High-Accuracy Molecular Docking." Chem., 2006.

14) H.M.Berman, et al "The Protein Data Bank" Nucleic Acids Research, (2000) .

15) D. Eisenberg et al, "VERIFY3D: assessment of protein models with three-dimensional profiles." Methods in enzymology, Vol. 277 (1997).

16) Gätke MR, et al, "Two novel mutations in the bche gene in patients with prolonged duration of action of mivacurium or succinylcholine during anaesthesia." Pharmacogenet Genomics. 2007.

17) Juan A. Contreras et al "Building of a three-dimensional model for the catalytic domain of hormone-sensitive lipase" The Journal of Biological Chemistry" Vol. 271, 1996.

18) "How Fat and Obesity Cause Diabetes" SANTA BARBARA, Calif., August 14, 2011, Sanford-Burnham Medical Research Institute.