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Research Article

Designing Inhibitors against Hexokinase 1 and Hexokinase 2 domain mutations of GCK and studying its association in Diabetes

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ABSTRACT

Diabetes mellitus, often simply referred to as diabetes, is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. In this study we analyzed the involvement of Hexokinase 1 and 2 domain of GCK (Glucokinase). Hexokinase catalyzes the phosphorylation of glucose intoglucose-6-phosphate (G6P) providing enough activation energy for the glycolytic process to start. A variety of tools and search engines are used to arrive the sequence, structure and function of protein. Disorder regions and surface properties, protein binding site was identified to dock specifically to the ligand. This protein was optimized to reach to maximum stability with minimum energy. Molecular dynamics simulation had been performed of target and ligand separately by applying periodic boundary condition, AMBER force field for protein and TRIPOSE force field for ligand. The molecules were energetically minimized by conjugate gradient method. Tripose SYBYL software have been used for molecular dynamic study. The drug discovery program includes finding all chemical compounds having potential to inactive the protein. Optimizing the candidate drugs and performing docking using bioinformatics software Argus Lab, Hex and Molegro.

Keywords: Diabetes mellitus; Hexokinase; Molecular dynamics; AMBER force field; Tripose SYBYL software; Drug discovery

INTRODUCTION

Glucokinase (GK) is a hexokinase isozyme, related homologously to at least three other hexokinases [1]. All of the hexokinases can mediate phosphorylation of glucose to glucose-6-phosphate (G6P), which is the first step of both glycogen synthesis and glycolysis. However, glucokinase is coded by a separate gene and its distinctive kinetic properties allow it to serve a different set of functions. Glucokinase has a lower affinity for glucose than the other hexokinases do, and its activity is localized to a few cell types, leaving the other three hexokinases as more important preparers of glucose for glycolysis and glycogen synthesis for most tissues and organs [2]. Because of this reduced affinity, the activity of glucokinase, under usual physiological conditions, varies substantially according to the concentration of glucose [3]. Mutations of the gene for this enzyme can cause unusual forms of diabetes or hypoglycemia. The Hexokinase 1 and Hexokinase 2 domain is 207 and 239 amino acids respectively in length, the position of Hexokinase 1 domain in GCK (glucokinase) started from 11 and ends with 218 residue of amino acid and the position of Hexokinase 2 started from 220 and ends with 459 residue of amino acid.

Hexokinase is an important enzyme that catalyses the ATP-dependent conversion of aldo- and keto-hexose sugars to the hexose-6-phosphate (H6P) [4]. The enzyme can catalyse this reaction on glucose, fructose, sorbitol and glucosamine, and as such is the first step in a number of metabolic pathways [5].The addition of a phosphate group to the sugar acts to trap it in a cell, since the negatively charged phosphate cannot easily traverse the plasma membrane.

The enzyme is widely distributed in eukaryotes. There are three isozymes of hexokinase in yeast (PI, PII and glucokinase): isozymes PI and PII phosphorylate both aldo- and keto-sugars; glucokinase is specific for aldo-hexoses. All three isozymes contain two domains [5]. Structural studies of yeast hexokinase reveal a well-defined catalytic pocket that binds ATP and hexose, allowing easy transfer of the phosphate from ATP to the sugar [6].

Vertebrates contain four hexokinase isozymes, designated I to IV, where types I to III contain a duplication of the two-

domain yeast-type hexokinases. Both the N- and C-terminal halves bind hexose and H6P, though in types I and III only the C-terminal half supports catalysis, while both halves support catalysis in type II. The N-terminal half is the regulatory region. Type IV hexokinase is similar to the yeast enzyme in containing only the two domains, and is sometimes incorrectly referred to as glucokinase. The different vertebrate isozymes differ in their catalysis, localisation and regulation, thereby contributing to the different patterns of glucose metabolism in different tissues [7].

Whereas types I to III can phosphorylate a variety of hexose sugars and are inhibited by glucose-6-phosphate (G6P), type IV is specific for glucose and shows no G6P inhibition. Type I enzyme may have a catabolic function, producing H6P for energy production in glycolysis; it is bound to the mitochondrial membrane, which enables the coordination of glycolysis with the TCA cycle. Types II and III enzyme may have anabolic functions, providing H6P for glycogen or lipid synthesis. Type IV enzyme is found in the liver and pancreatic beta-cells, where it is controlled by insulin (activation) and glucagon (inhibition). In pancreatic beta-cells, type IV enzyme acts as a glucose sensor to modify insulin secretion. Mutations in type IV hexokinase have been associated with diabetes mellitus.

In our work we predict the tertiary structure of the protein (Glucokinase) and found the fold recognition that is functional region of the protein, next we found the disorder region in the domain which is responsible for disease, by tools (RONN, Disopred, DisEMBL, GLOBPLOT 2) and search for the binding site by (Q site finder, pocket finder, castp) to bind the drug compound at particular amino acid by docking software's like (Argus lab, Hex and Molegro virtual docker). In this report we identify a single amino acid which lies in the in Hexokinase domain range which have mutation, by this we can say that this amino acid is responsible for unusual forms of diabetes or hypoglycemia..

MATERIALS AND METHOD

Disorder prediction

Disorder region in the protein sequence were predicted by the tools of bioinformatics like (RONN, Disopred, DisEMBL, and GLOBPLOT 2). Which show the disorder or mutational region in the protein sequences [8,9,10,11].

Binding site and Active site identification

The binding site or active site identification is the method of finding the site where the ligand binds, for binding site prediction we used Q site finder, pocket finder, and Castp and tools by which we select the best binding site for the drug. [12,13,14]

Energy Minimization

Energy minimization was carried out of both receptor and ligand molecule. Energy minimization was done by using AMBER force field and applying steepest descent method to get the optimal energy of the molecules. The energy minimization of ligand was done by using Tripos force field. [15]

Docking

Docking is a method which predicts the preferred orientation of one molecule to a record when bound to each other to form stable complex knowledge of the preferred orientations in turn may be used to predict the binding strength of association or binding affinity between two molecules. Docking is frequently used to predict the binding orientations of small molecules drug candidates to protein targets in order to in turn predict the affinity and activity of the small molecule. The receiving molecule that primarily binds to a small molecule or another protein or a nucleic acid called receptor. A molecule that forms the complementary partner in the docking process called ligand, we used several software for docking i.e. Arguslab, molegro, Hex. [16,17]

RESULTS AND DISCUSSION

Sequence retrieval

The protein sequence was retrieve from the GeneCard by selecting the sequences from whole involved sequences in the diabetes mellitus disease these sequences are screened and we found the sequence which is playing the major role in disease i.e. GCK.

Domain Analysis

Domain identification was done by using bioinformatics database (SMART) and found Hexokinase 1 and Hexokinase 2 domain which is 207 and 239 amino acid respectively residues long, the position of Hexokinase 1 domain in GCK (glucokinase) started from 11 and ends with 218 residue of amino acid and the position of Hexokinase 2 started from 220 and ends with 459 residue of amino acid.

Disorder Prediction

Disorder prediction in the protein was done by the tools (RONN, Disopred, DisEMBL, and GLOBPLOT 2). The results show the major disorder region in the protein sequence, the disorder region which lies in the domain range were selected for docking the candidate drug.

RONN is the the bio-basis function neural network technique applied to the detection of natively disordered regions in proteins. After using RONN we found a graph (Figure 1) in which we found the higher peak of disorder region in the protein sequence at 55 amino acid residue which is lies between the domain ranges.

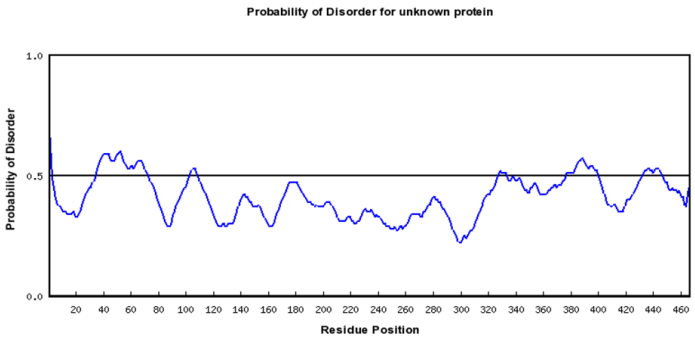

Figure: 1 Higher peak of disorder region in the protein sequence at 55 amino acid residue.

DisEMBL is a public web server for predicting disorder in proteins. After using DisEMBL we found a graph (Figure 2) in which we see the higher peak of disorder region in the protein sequence which is lies between the domain ranges.

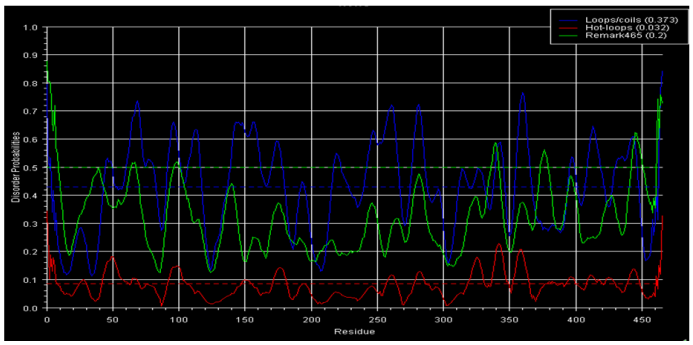

Figure: 2 Higher peak which comes in domain range (region has higher disorder in the protein sequence)

Disopred tool is used for finding the disordered region in the protein sequence. Figure 3 represent a graph between sequence number and disorder probability. In this graph we found that higher peak of disorder region in the protein sequence which lies between domain ranges.

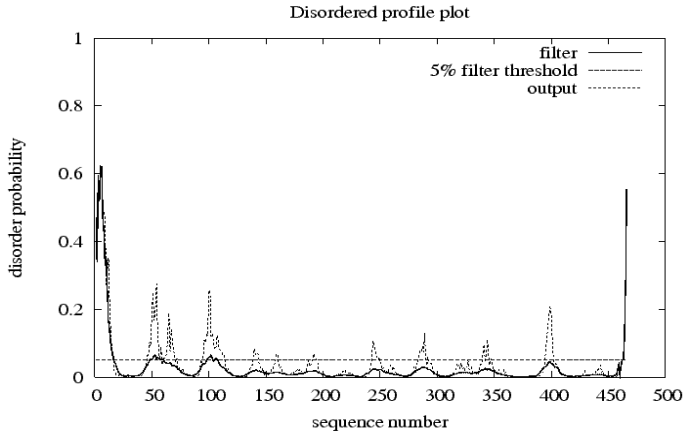

Figure: 3 Higher peak of disorder region in the protein sequence.

Binding site and Active site identification

The binding site and active site identification is the method of finding the site where the ligand binds. Using bioinformatics tools and approach we found the site which is good for ligand to bind and it comes in the domain region which concludes that the mutation in that site is responsible for diabetes mellitus, Q site finder, pocket finder tools are used for this approach. All three tools identified the same binding site SER (Serine) in the protein sequence. This amino acid residue is targeted with the drug compound to cure mutation.

Energy minimization of target

Energy minimization was carried out by using sybyl software. AMBER force filed was applied and energy minimization was done by steepest descent method. Energy breakdown of the initial energy calculation of receptor molecule presented in Table 1 and energy breakdown of the optimized energy of receptor presented in Table 2.

Table 1. Initial energy of the target

Bond Stretching Energy	675.842 kcals/mol
Angle Bending Energy	705.787 kcals/mol
Torsional Energy	462.129 kcals/mol
Out of Plane Bending Energy	26.738 kcals/mol
1-4 van der Waals Energy	-149.226 kcals/mol
van der Waals Energy	-2235.695 kcals/mol
1-4 Electrostatic Energy	45874940.417 kcals/mol
Electrostatic Energy	785546300.221 kcals/mol
Total Energy	831420726.214 kcals/mol

Table 2. Optimized energy of receptor

Bond Stretching Energy	266.42 kcals/mol
Angle Bending Energy	75.787 kcals/mol
Torsional Energy	162.129 kcals/mol
Out of Plane Bending Energy	26.17 kcals/mol
1-4 van der Waals Energy	-95.206 kcals/mol
van der Waals Energy	-2235.695 kcals/mol
1-4 Electrostatic Energy	2040.417 kcals/mol
Electrostatic Energy	6300.221 kcals/mol
Total Energy	-10726.784 kcals/mol

Tripose force field is used for ligand energy optimization. Energy breakdown of the initial energy of the ligand molecule listed in Table 3 and optimized energy of the ligand presented in Table 4.

Table 3. Initial Energy of the Ligand Molecule.

Bond Stretching Energy	16.605 kcals/mol
Angle Bending Energy	239.857 kcals/mol
Torsional Energy	0.526 kcals/mol
Out of Plane Bending Energy	0.000 kcals/mol
1-4 van der Waals Energy	4901689.943 kcals/mol
van der Waals Energy	28.747 kcals/mol
1-4 Electrostatic Energy	0.000 kcals/mol
Electrostatic Energy	0.000 kcals/mol
Total Energy	4901975.678 kcals/mol

Table 4. Optimized energy of the ligand.

Bond Stretching Energy	0.412 kcals/mol
Angle Bending Energy	120.822 kcals/mol
Torsional Energy	0.323 kcals/mol
Out of Plane Bending Energy	0.000 kcals/mol
1-4 van der Waals Energy	-0.107 kcals/mol
van der Waals Energy	-0.067 kcals/mol
1-4 Electrostatic Energy	0.000 kcals/mol
Electrostatic Energy	0.000 kcals/mol
Total Energy	121.384 kcals/mol

Docking

Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex, Applying different approaches and software of bioinformatics like (Argus lab, HEX, Molegro Virtual docker) we use to dock the inhibitor compounds to the protein binding site where the compound binds properly and show the result. Figure 4 shows the hex docking result in which we found Etotal, Eshape and E force value of Isoamile nitrite which is -139.44(kcal/mole), -139.44 and 0.00 respectively.

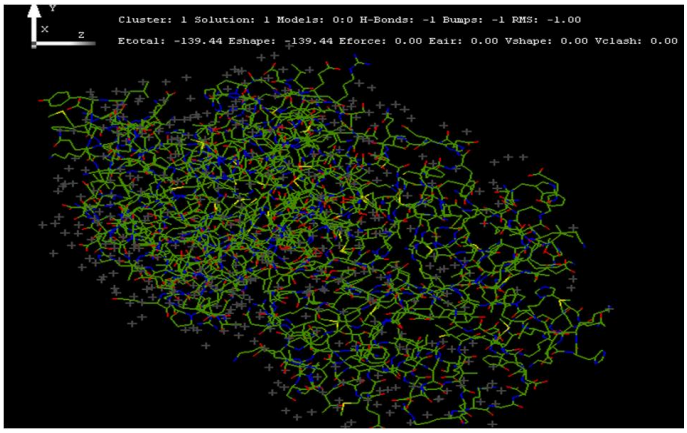

Fig 4. Hydrogen bond interaction and Etotal, Eshape, Eforce.

Figure 5 shows the hydrogen bond interaction and short contacts between ligand and the target, involving the interaction between the amino acids, which gives Mol doc Score of Isoamile nitrite is -47.2898.

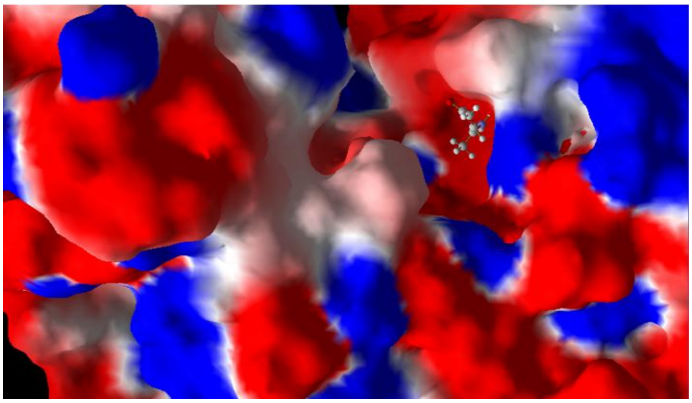

Figure: 5 Hydrogen bond interaction and short contacts between ligand and the target.

After dock the ligand with receptor using Argus lab software we found the docking energy of isoamile nitrile is -6.90901.

CONCLUSION

In our study we see that the Hexokinase 1 and Hexokinase 2 domain which lie in GCK protein is responsible for proteins control, mediate phosphorylation of glucose to glucose-6-phosphate (G6P), which is the first step of both glycogen synthesis and glycolysis. Here we identified the mutational region in the protein sequence. Further we find the binding region, the region to which our drug bind and found the single amino acid Serine (SER) which is at 55 position in protein sequence. While docking we target the domain mutational region in which the amino acid SER is present to that we bind the drug and observed that the drug Isoamyl nitrite has good hydrophobic effect based on LogP value which bind with target sequence showing the less energy, this show that the drug is best to correct the disorder in the protein sequence, the docking result were identified by different docking software like Argus lab, molegro, Hex. Finally we conclude that the drug is showing the higher binding affinity between the ligand and protein so we can say that this drug is best to correct the mutation and therefore **Isoamyl nitrite** (Figure 6) **(**CID: CHEBI: 2691, Molecular weight: 117.148) can be used in future as a diabetes drug.

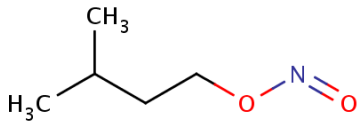

Figure: 6 Isoamyl nitrite

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