



Gene Analysis and Docking for GCGR Gene involved in Non Insulin Dependent Diabetes Mellitus (NIDDM)

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Received: 15th November 2014, Accepted: 17th December 2014, Published: 1st March 2015

Abstract:

Diabetes mellitus is a disorder of carbohydrate metabolism characterized by impaired ability of the body to produce or respond to insulin and thereby maintain proper levels of sugar (glucose) in the blood. Long since the disease was studied, insulin has gained a lot of importance in the disease occurrence and treatment. However, there are several other proteins that are yet not studied to the extent but are known to have an important role in the disease. Some of the proteins involved in Diabetes mellitus include BDNF, BCHE, GCGR etc.,. The current work was undertaken with an aim to analyze the GCGR gene (glucagon receptor). It is a G protein coupled receptor for glucagon hormone and plays a central role in the blood glucose regulation. The current work involves the collection of the Diabetic blood samples and extraction of the DNA using the Buncce Method. The extracted DNA was purified and visualized in AGE. Primers were designed for the amplification of the GCGR gene and the samples were sent for sequencing, which include both patient and Normal DNA samples. The sequences were then used for Multiple sequence alignment and the SNP regions in the Patient samples were identified. The work was extended to the Protein Analysis which included protein 3D structure collection and developing a potential drug to inhibit the complications of Type II Diabetes. The chemicals were collected from the Pubchem database and were screened for several parameters like Drug likeliness, Ambiguity and Toxicity. All the selected chemicals were then used to dock with the 3D structure of the GCGR protein using the HEX Software. Based upon the energy values of the docking pairs, the best docked ligand with lowest docking energy can be finalized.

Keywords:

GCGR, SNP, Docking, Ambiguity, Drug likeliness, HEX, Multiple Sequence alignment.

Introduction:

Diabetes is a major cause of morbidity and mortality, though these outcomes are not due to the immediate effects of the disorder. They are instead related to the diseases that develop as a result of chronic diabetes

mellitus. These include diseases of large blood vessels (macro vascular disease, including coronary heart disease and peripheral arterial disease) and small blood vessels (micro vascular disease, including retinal and renal vascular disease), as well as diseases of the nerves. Type II diabetes is far more common than type I diabetes, accounting for about 90 percent of all cases. The frequency of type II diabetes varies greatly within and between countries and is increasing throughout the world. Most patients with type II diabetes are adults, often older adults, but it can also occur in children and adolescents. There is a stronger genetic component to type II diabetes than to type I diabetes. For example, identical twins are much more likely to both develop type II diabetes than to both develop type I diabetes, and 7 to 14 percent of people whose mother or father has type II diabetes will also develop type II diabetes; this estimate increases to 45 percent if both parents are affected. In addition, it is estimated that about 40 percent of the Pima Indian population in Arizona has type II diabetes, whereas in the entire United States it is estimated that more than 10 percent of the population has type II diabetes.

Glucagon is a pancreatic hormone produced by cells in the islets of Langerhans. Glucagon is a 29-amino-acid peptide that is produced specifically by the alpha cells of the islets. It has a high degree of similarity with several glucagon-like peptides that are secreted by cells scattered throughout the gastrointestinal tract. Glucagon secretion is stimulated by the ingestion of protein, by low blood glucose concentrations (hypoglycemia), and by exercise. It is inhibited by the ingestion of carbohydrates, an effect that may be mediated by the resultant increase in blood glucose concentrations and insulin secretion. Glucagon strongly opposes the action of insulin; it raises the concentration of glucose in the blood by promoting glycogenolysis, which is the breakdown of glycogen (the form in which glucose is stored in the liver), and by stimulating gluconeogenesis, which is the production of glucose from amino acids and glycerol in the liver. By increasing the concentration of glucose in the bloodstream, glucagon plays a critical role in maintaining blood glucose concentrations during fasting and exercise.

Materials and Methods:

Collection of the Diabetic and Normal Blood Samples:

In order to analyze the GCGR gene among the diabetic and normal patients, their blood samples have to be collected. The blood samples were collected from the nearby Diagnostic centre and hospital. All the samples were received in the EDTA vials to preserve the sample for the analysis.

Extraction of the DNA from the Blood Samples and AGE:

The DNA was extracted from the samples as per the basic protocol and all the samples were precipitated with ethyl alcohol overnight at 4⁰C. After the incubation period the sample was centrifuged and the pellet was washed with Ethyl Alcohol for the elimination of lipid and protein impurities. The DNA extracted was further run in Gel Electrophoresis with 8% of agarose. The Bands thus produced were observed against UV Trans Illuminator.

Sequence retrieval of GCGR gene from NCBI and Primer Designing:

NCBI public database was used to retrieve the sequence of the GCGR gene. The gene was 2,053 bp in length. The Primers were designed for this Gene using **Primer Blast**. The forward and reverse primers were obtained. These primers were further used for the amplification of the samples on PCR.

PCR Amplification for the GCGR genes:

All the samples of Diseased as well as normal were used for PCR based amplification using the Primers designed by PRIMER BLAST. The PCR mixture was made using the DNA sample, dntp's, RNase free water, MgCl₂, Taq polymerase etc. The PCR machine was set for 30 cycles and the required gene was amplified.

Post PCR AGE:

To confirm the amplification of the DNA after the PCR step Gel Electrophoresis was performed again including the Ladder for reference. The successful amplification of the gene was indicated by the prominent band in the Gel under UV Transilluminator.

Sequencing of the samples:

The DNA samples after amplification were sent for sequencing. the protocol employed for sequencing is the Sanger's method of DNA hydrolysis.

The sequences thus obtained were further assembled and used for further analysis.

In Silico Sequence Analysis:

The gene sequences thus obtained were used further for analysis based on *in silico* tools as follows:

Multiple Sequence Alignment using SDSC Biology Workbench and Identification of the SNP's:

To compare the sequences of the samples obtained Multiple sequence alignment has to be performed. SDSC Biology work bench has been used for this purpose. The tool CLUSTAL W available in the Gene Analysis tools has been employed. Based on the Sequence alignment the regions of SNP and Conservation can be observed.

Protein Structure download form RCSB Protein Data Bank:

To obtain the 3D structure of the protein that was coded by the GCGR gene RCSB PDB was used. The searching was based on basic BLST technique. All the structures that showed the relation with the GCGR word was retrieved and the PDB ID 2L63 was selected as the structure representative of the GCGR protein. This was further used for the next analysis.

Chemical Analysis and Drug Identification:

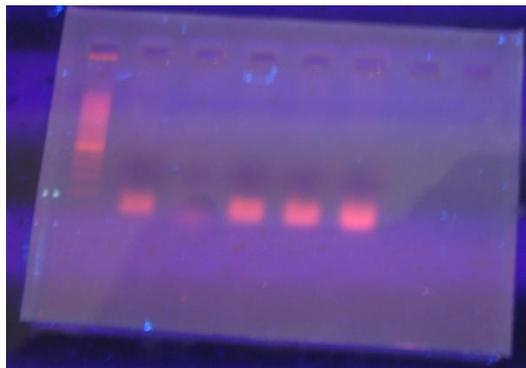
In order to act against the adverse effects caused by the GCGR protein, chemicals are used as drugs after through screening. The chemicals were collected from Pubchem Database. All the chemicals were screened for their Drug likeliness based on Lipinski rule of 5. The selected chemicals were used for the ambiguity testing using Dundee prodrgr server. The selected proteins with low ambiguity are further screened for toxicity using Mol inspiration and finally the best chemicals are selected.

Docking of the Selected Ligand with the Receptor Protein:

The chemical compound that was selected in all the screening steps was further downloaded and used for the docking with Protein in Hex Software. After docking based on the docking energy best chemical has to be selected, which can be further used for drug compound preparation and medication.

Results and Discussion:

Fig 1: Agarose Gel Electrophoresis for DNA Banding:



The above figure shows the Gel picture of 5 samples of DNA and the first well shows the ladder used for reference.

Primer Designing for GCGR gene using BLAST P:

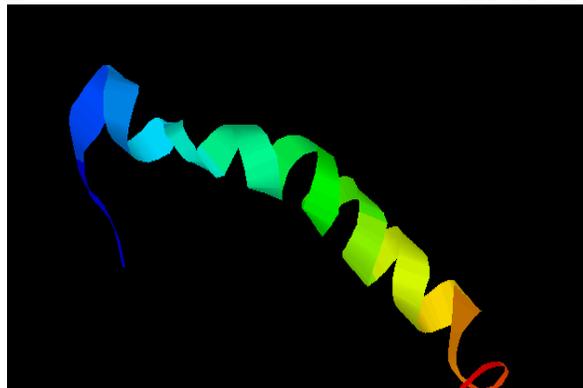
Table 1: Showing the Forward and Reverse Primers designed

Forward Primer	TAGATTGGCTGAGAGCCCCT	Plus Strand
Reverse Primers	CGGTGACCACTTGAGCTCTT	Minus Stand

3D Structure prediction based on RCSB Protein data Bank:

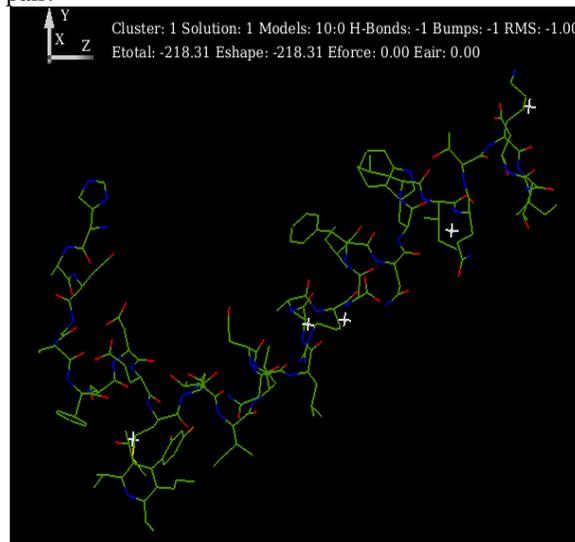
To retrieve the 3D structure of the protein the gene name is used in RCSB PDB. Based upon the result the best ID selected as the protein structure representative was **2L63**. The structure was retrieved from PDB and used for further analysis.

Fig 2: The figure shows the 3D structure of GCGR protein as obtained from RCSB PDB and viewed in RASMOL



Docking of the Selected Ligand with the Receptor in HEX:

Based upon the screening result the best ligand selected was **glucagon receptor antagonists-3** which was used to dock with the GCGR protein **2L63** using **HEX** Software. The energy obtained for this docking pair was -218.31 which indicates a good docking pair.



Conclusion:

In the above work gene analysis of GCGR was performed after obtaining the sequence from the blood samples of the diseased patients. The sequence analysis was performed and the protein structure selected as the structure representative was **2L63**. The structure was selected for docking. The chemical that was selected for the docking with the receptor was **glucagon receptor antagonists-3**. The docking was performed in HEX software and the docking energy obtained was -218.31. Thus indicates that the ligand can be best suited as the drug to act against the adverse effects of the disease Diabetes.

Biobibliography:

1. Williams textbook of endocrinology (12th ed.). Philadelphia: Elsevier/Saunders. pp. 1371–1435.
2. <http://www.ncbi.nlm.nih.gov/gene/2642>
3. <http://www.ncbi.nlm.nih.gov/protein/AAH05278.1>
4. Lengauer T, Rarey M (1996). "Computational methods for biomolecular docking". *Curr. Opin. Struct. Biol.* 6 (3): 402–6. doi:10.1016/S0959-440X(96)80061-3. PMID 8804827.

5. Vijan, S (March 2010). "Type 2 diabetes". *Annals of Internal Medicine* pp-152
6. "Diabetes mellitus". *Merck Veterinary Manual, 9th edition (online version)*.
7. Reece J, Campbell N (2002). *Biology*. San Francisco: Benjamin Cummings
8. LILJENQUIST, JOHN E. et al "Effects of Glucagon on Lipolysis and Ketogenesis in Normal and Diabetic Men". *J Clin Invest*. 53 (1): 190–7.
9. <http://www.ncbi.nlm.nih.gov/pubmed/25537454>
10. <http://www.ncbi.nlm.nih.gov/pubmed/25547241>
11. <http://www.nlm.nih.gov/medlineplus/diabetes>
12. <http://intranasal.net/glucagon/default.htm>
13. <http://www.medicalnewstoday.com/info/diabetes/>
14. <http://science.marshall.edu/murraye/links%20for%20students/samantha%20qiagin%20method.pdf>
15. <http://learn.genetics.utah.edu/content/labs/gel/electrophoresis/>