Int J Pharm Bio Sci 2012 Oct; 3(4): (B) 493 - 499

Research Article **Bioinformatics**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

MOLECULAR DYNAMICS SIMULATIONS OF MODY2 MUTATED GLUCOKINASE STRUCTURES REVEALED SIGNIFICANT CONFORMATIONAL VARIATIONS EXPLAINING REASONS FOR HYPERGLYCEMIC CONDITION

Y. NANDA KUMAR[1], K. KALPANA[1], K. VENKATESWARA SWAMY[2], P.V.G.K.SARMA[3] AND M. BHASKAR[1*]

[1]Division of Animal Biotechnology, Department of Zoology, Sri Venkateswara University, Tirupati, Andhrapradesh, India, 517502.
[2]Department of Bioinformatics and Computer Science, Dr.D.Y.Patil Biotechnology and Bioinformatics Institute, Dr. D. Y. Patil Vidya peeth, Pune, India, 411033.
[3]Department of Biotechnology, Sri Venkateswara Institute of medical sciences, Tirupati, Andhrapradesh, India, 517507.

ABSTRACT

Glucokinase (GK) involves in the phosphorylation of Glucose in pancreatic β-cells and liver to maintain normal blood glucose levels. Inactivating and activating mutations in GK gene can influence the affinity for glucose and leads to altered glucose levels in blood causing Maturity Onset Diabetes of the Young2 (MODY2) condition. In this study, impact of 42 MODY2 mutations on GK conformation was studied using molecular dynamics study. Simulations were carried out for intact and mutated GK structures and the correlation of their energy and RMSD (Root Mean Square Deviation) values showed significant differences. The PDBSum analysis of simulated structure revealed the clear variation in the conformation of mutated GK structures where they showed increased γ-turns, decreased β-turns and more helix-helix interactions with respect to intact GK. Such a conformational variations could affect the activity of GK and may results in hyperglycemic condition in MODY2 patients.

KEYWORDS: Glucokinase, MODY2, Molecular Dynamics, RMSD.

M. BHASKAR
Division of Animal Biotechnology, Department of Zoology, Sri Venkateswara University, Tirupati, Andhrapradesh, India, 517502

INTRODUCTION

MODY2 is a type2 diabetic condition where the increased blood glucose levels are observed due to the mutations in GK gene and the mutations are referred as MODY2 mutations. It is an autosomal dominant inherited type of diabetes with significant genetic heterogeneity. Hundreds of MODY2 mutations have been reported so far in GK gene[1]. The severity of the disease can be different for different types of mutations within the gene. It has been demonstrated that many of these mutations cause a decrease in Vmax, an increase in the glucose $S_{0.5}$ and or a change of the ATP Km, either present alone or in combination. The phenotype of the specific type of MODY2 mutation and its location within the gene varies with the disease severity. The mutations that are confined to the domain regions elicit more severity of the diabetic condition[2]. Kesavan *et al*., have provided the first direct evidence that certain amino acid substitutions may result in protein instability as the singular cause of the enzyme defect [3]. It has been noticed that the kinetic variations of glucokinase by MODY2 mutations is due to structural variations raised in the conformation of the protein. GK shows a larger domain and smaller domain in between the active site is located [4]. The catalysis process is mediated by open-closed conformational equilibriums of the active site, which will be affected by the mutations. Any mutations in the smaller domain results in conformational change and there by affect glucose binding and also catalysis[5]. The location of the mutation may be playing a role in eliciting the functional defects of the protein, but whatever the location of mutation, it will results in the variation of conformations and energy levels. These variations may show their impact on the functionality of the protein where its activity may be increased or decreased[6]. The study of these mutations and their impact on the conformations is an important point under consideration, but the experimental evaluation is a limiting factor due to expensive and tedious tasks. This can be overcome by the molecular dynamics approach, a popular computational tool, have the ability to identify the impact of mutations on the energy levels and RMSD fluctuations of proteins within a less time period[7]. It is a computational simulation technique where the molecule is allowed to interact for a period of time to give a glance of the atoms and bonds that can be reflected on the energy levels and conformations of the molecule along the time period. All the trajectories of the molecule during the simulations are determined by Newton's equations of motion in which their energies are defined by the specified molecular mechanics force field[8,9]. Here in the present study we applied this molecular dynamics approach to carry out a comparative study on the intact and mutated GK structures to explain the impact of mutations on the conformation and energy levels. The dynamic behavior of the GK structures during simulations gave a better understanding on the correlation of its structure, function and dynamic implications. We observed drastic variations in the conformations of the mutated structures with respect to intact structure and these variations were reflected in the total energies and RMSD values also. All such a variations in the GK structure may results in the alterations of its enzymatic activities where it is unable to phosphorylate the glucose molecule, there by increasing blood glucose levels causing hyperglycemic condition in the patients with MODY2 mutation.

MATERIALS AND METHODS

The X-Ray crystallographic structure of GK (PDB ID: 3F9M) was retrieved from Protein Data Bank (http://www.rcsb.org/pdb/home/home.do) which is a huge repository of three dimensional structures of macromolecules[10]. The water molecules and hetero atoms present in the system were removed, the protein structure

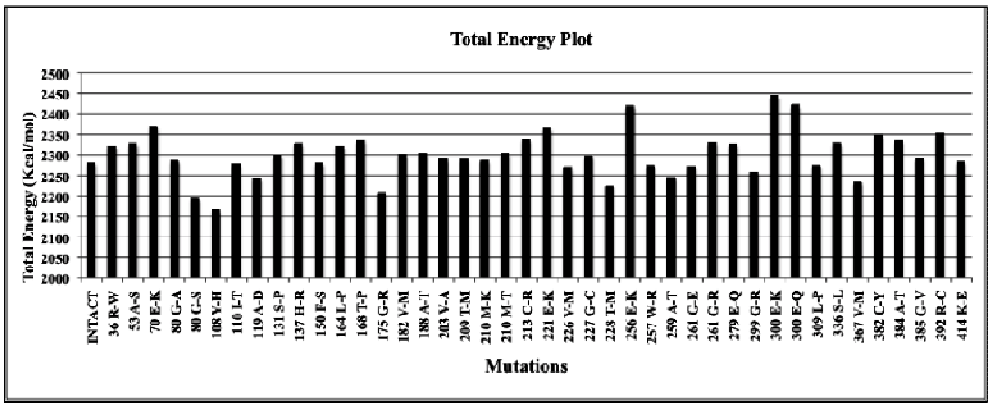
was protonated and polar hydrogens were added. Energy minimization was performed by applying Polak Ribiere conjugate gradient algorithm at RMS gradient of 0.1 for 3000 maximum cycles. Once the structure was relaxed, a 10 pico seconds (ps) molecular dynamics simulation was performed to heat the system from 0K to 300K. Simulations were carried out by applying AMBER99 force field and the coordinates of all atoms were allowed to move freely during molecular dynamics simulations. To bring the system to perfect density, a 10 nano seconds (ns) molecular dynamics run was carried out. A constant temperature was maintained with a bath relaxation time of 0.1ps. For the analysis purpose each trajectory was trapped for every 0.1ps. Average values were calculated for potential, kinetic and total energies along with temperature fluctuations. HyperChem 7.5 software tool, a sophisticated molecular modeling environment which works by uniting with quantum chemical calculations, molecular mechanics and dynamics was used for the molecular dynamics study of GK[11].

A total of 42 MODY2 associated mutations which are already reported were obtained from SWISS PROT data base (http://expasy.org/sprot/) from the GK entry (SWISS PROT ID: P35557)[12]. Energy minimization and molecular dynamics study was carried out as explained above by introducing 42 mutations individually into the intact GK structure. Each time the simulated mutated structure and its energy values were saved without fail. Finally a 43 simulated conformations (1 intact + 42 mutated) were obtained from molecular dynamics study. The total energy values and their RMSD values were plotted as bar charts to observe the variations with reference to intact GK structure. All the 43 simulated structures were submitted to PDBSum which can provide an at-a-glance overview of macromolecular structure[13]. PDBSum analysis allows observing the conformational variations that aroused due to introduction of each mutation with respect to intact GK structure. The simulated mutated structures were superimposed with intact structure to get a clear insight about the conformational fluctuations, especially in substrate binding regions.

RESULTS AND DISCUSSION

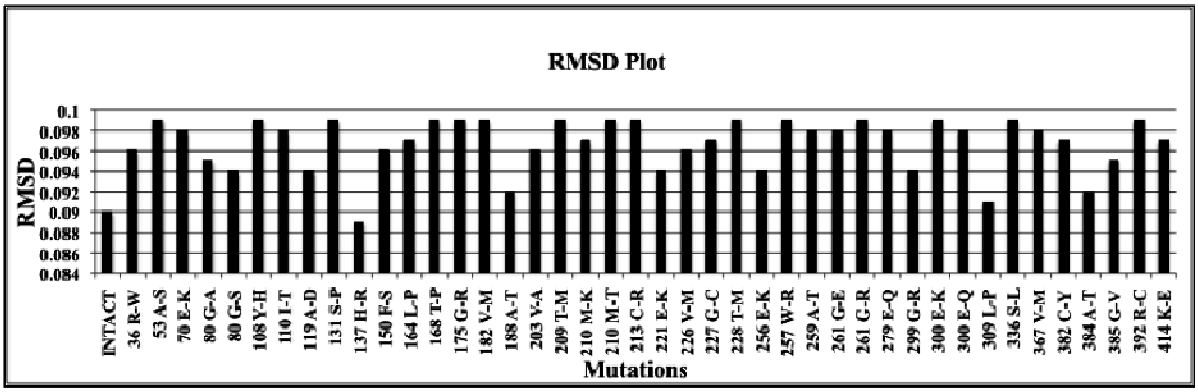
To assess the stability of the simulations the RMSD of the Cα atoms of all the mutated structures with respect to intact structures were monitored. The molecular dynamics results showed that stable trajectories were obtained by the end of simulations. The stabilized trajectories of intact GK structure and 42 mutated GK structures were observed for their total energy and RMSD variations after molecular dynamics simulations. The intact GK is showing the energy of 2280.14 Kcal/mol and using this value as reference the energy values of 42 mutated structures were compared to observe their deviations from the normal energy levels. Among these 42 mutated structures, 108 Y – H mutated GK structure is showing the least total energy of 2164.84 Kcal/mol and 300 E – K mutated GK structure is showing highest total energy of 2442.54 Kcal/mol (Figure 1).

Figure 1
The Total energy plot of glucokinase structures.



Bar chart showing the impact of mutations on the total energy of GK structure. The first bar is showing the total energy of intact GK (2280.14 Kcal/mol) and the remaining bars showing the total energies of mutated GK structures. The intact GK structure is showing an RMSD value of 0.090 and among the mutated GK structures, a least RMSD of 0.089 was found with 137 H – R mutated GK and highest RMSD of 0.099 was found with 53 A – S, 108 Y – H, 131 S – P, 168 T – P, 175 G – R, 182 V – M, 209 T – M, 210 M – T, 213 C – R, 228 T – M, 257 W – R, 261 G – R, 300 E – K, 336 S – L and 392 R – C mutated GK structures (Figure 2).

Figure 2
RMSD plot of Glucokinase structures after molecular dynamics simulations



Bar chart showing the impact of mutations on the RMSD of GK Structure. First bar is showing the RMSD of intact GK structure (0.090) and the remaining bars showing the RMSD of mutated GK structures. The mutations showing the values beyond the range of intact GK values could explain that those are the most effective mutations and the values which are nearer are less effective bringing the variations in the reactivity of mutated structures. At the lowest energy rates the molecule remains stable and is inert so as to avoid its interaction with other molecules like substrate and Co factors. Even under high energy conditions also the molecules is unable to interact with its substrates and Co factors[14,15]. Here, the mutated GK structures that are showing lowest and highest energies than intact GK may be unable to interact with glucose and ATP molecules and there may be no phosphorylation of glucose resulting in high glucose concentrations.

These mutations showed their effect not only on the total energy and RMSD of the GK but also on the secondary structure conformations. The PDBSum analysis revealed the conformational variations in the GK structures that aroused due to each mutation. The comparison of secondary structure conformations of intact and mutated GKs showed variations in number of β bulges, strands, helices, helix-helix interactions, β-turns and a major difference in γ turns. Variations were not seen with the number of sheets, beta alpha beta units and β-hairpin elements (Table 1).

Usually γ – turns are formed by hydrogen bonds between the carbonyl oxygen of one residue and amide hydrogen of another residue a head. Milner *et al* explained a concept about these γ-turns that the formation of inverse γ-turns in a protein produces kinks in the structure and makes the direction of chain reversal resulting in instability of the protein[16]. Based on this concept it can be assumed that as there is a drastic increase in the number of γ-turns in the above explained mutated structures, these γ-turns may cause the conformational variations bringing instability of the protein. All these conditions may cause the altered catalytic activity of GK resulting in improper phosphorylation rates finally resulting in hyperglycemic condition.

Table 1

PDBSum analysis showing the variations in secondary structural confirmations of intact and mutated GK structures. The first row corresponds to the intact structure and the remaining rows correspond to the mutated structures.

S.No	Mutation	Sheets	Beta Alpha Beta Unit	Beta Hairpins	Beta bulges	Strands	Helices	Helix-Helix Interactions	β turns	γ turns
1.	Intact	3	1	5	5	13	20	24	34	3
2.	36_R-W	3	1	5	4	13	22	39	33	16
3.	53_A-S	3	1	5	4	13	22	40	29	16
4.	70_E-K	3	1	5	4	14	22	40	29	15
5.	80_G-A	3	1	5	4	13	22	40	29	16
6.	80_G-S	3	1	5	4	13	22	34	30	15
7.	108_Y-H	3	1	5	4	13	23	39	30	18
8.	110_I-T	3	1	5	4	13	22	35	31	16
9.	119_A-D	3	1	5	4	13	22	40	30	18
10.	131_S-P	3	1	5	4	13	23	39	29	15
11.	137_H-R	3	1	5	4	13	22	34	31	17
12.	150_F-S	3	1	5	4	13	22	40	31	16
13.	164_L-P	3	1	5	4	13	22	40	31	15
14.	168_T-P	3	1	5	4	13	23	40	30	17
15.	175_G-R	3	1	5	4	13	23	34	30	15
16.	182_V-M	3	1	5	3	13	23	39	30	17
17.	188_A-T	3	1	5	4	13	23	39	28	16
18.	203_V-A	3	1	5	4	14	22	39	32	16
19.	209_T-M	3	1	5	4	14	22	39	29	16
20.	210_M-K	3	1	5	4	13	22	40	31	16
21.	210_M-T	3	1	5	5	13	22	35	30	15
22.	213_C-R	3	1	5	5	13	22	38	30	16
23.	221_E-K	3	1	5	5	13	22	40	34	13
24.	226_V-M	3	1	5	4	13	22	38	29	15

25. 227_G-C	3	1	5	4	13	23	39	30	14
26. 228_T-M	3	1	5	4	13	23	34	30	16
27. 256_E-K	3	1	5	4	13	22	40	31	13
28. 257_W-R	3	1	5	4	13	23	33	31	16
29. 259_A-T	3	1	5	4	13	22	40	29	18
30. 261_G-E	3	1	5	4	13	23	38	30	16
31. 261_G-R	3	1	5	4	13	22	39	31	15
32. 279_E-Q	3	1	5	4	13	23	39	29	16
33. 299_G-R	3	1	5	4	13	23	39	31	15
34. 300_E-K	3	1	5	4	13	22	35	31	19
35. 300_E-Q	3	1	5	4	13	22	34	31	14
36. 309_L-P	3	1	5	4	13	23	39	30	17
37. 336_S-L	3	1	5	5	13	23	39	28	14
38. 367_V-M	3	1	5	4	13	23	35	30	16
39. 382_C-Y	3	1	5	5	13	23	40	27	14
40. 384_A-T	3	1	5	4	13	22	40	29	16
41. 385_G-V	3	1	5	4	13	22	39	33	16
42. 392_R-C	3	1	5	4	13	22	40	30	15
43. 414_K-E	3	1	5	4	13	22	37	31	17

CONCLUSION

The conformational fluctuations that aroused in the structure of GK are due to the mutations which may alter its affinity for binding with Glucose and ATP. This study had best explained the conformational variations of structure. Finally, it provided a strong reason for the affinity changes in terms of both energy and RMSD.

ACKNOWLEDGEMENTS

This work is supported by INSPIRE Division, Department of Science and Technology, Govt. of India. We acknowledge gratefully for providing DST INSPIRE Fellowship.

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