Homology Modelling and Docking Studies of p85-alpha a subunit of Phosphatidylinositol 3-kinase with Anti-diabetic Compounds

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Abstract: *The p85α regulatory subunit of phosphoinositide 3-kinase (PI3K), a key mediator of Insulin's metabolic actions. This gene plays a major role in the mediation of insulin- stimulated glucose disposal. The understanding of this enzyme might significantly increase the knowledge on the design of novel anti-diabetic compounds. Thus in the present study, the 3D structure of PI3K-p85alpha was modelled by using Modeller9v9 and the structure was validated by checking its stereo chemical parameters through PROCHECK, Verfiy3D, and ERRAT at SAVES server. Further, the docking studies with the ten widely used anti-diabetic compounds were carried out by using FlexX suite. The docking interactions revealed that the Phenformin (CID-8249) is having strong interaction of -24.1844 kJ/mol and the lowest interaction of -13.9702 kJ/mol was observed for Saxagliptin. The docking interactions determined that Phenylalanine (Phe59) and Serine (Ser60) amino acids are crucial for H bond interactions and the Hydrophobic interactions (Non bonded) is flavoured by Tyrosine (Tyr 83) residues in the active site of PI3K alpha. The finding of this work might enhance the understanding of PI3K alpha resistance and also helps in design of novel anti-diabetic compounds.*

Keywords : *Phosphoinositide 3 – kinase, p85 alpha, Homology Modeling, Docking studies, anti-diabetic compounds.*

1. INTRODUCTION

It is to be noted with astonishment that diabetes is attracting global importance, as it is rocking the whole world as a non-infections epidemic or even pandemic. This is virtually true with reference to Indian sub- continent, which has been declared by the world health Organization as the, "Diabetes capital of the world". Among the many surprising issues about diabetes in the recent past, one is the "increasing emergence of type-2 diabetes in childhood and adolescence. "Because, till recently, it was a popular belief that diabetes is seen in subjects under the age group of 30 years is always type-1. The term, 'adult-onset diabetes', previously described for type-2 diabetes, is also correct is the sense that the type-2 form of diabetes frequently goes undiagnosed for many years because such patients develop hyperglycemia very gradually even with normal or elevated insulin levels. At least initially, and often throughout their life time, these individuals do not need insulin treatment to survive [1]. Those human subjects, until they are diagnosed as type-2 diabetic, are categorized as Insulin resistance group. The clinical spectrum of insulin resistance is quite broad, and may include those patients with diabetes mellitus (type 2) who require insulin and who continue to have hyperglycemia despite large doses of exogenous insulin and patients who maintain near normal blood glucose levels through marked elevations in endogenous insulin secretion. The latter groups of patients are the real insulin resistance groups whose etiology may be mostly due to the mutations of insulin-receptor gene. It is with this baseline concept that the present study was taken up.

Phosphoinositide 3 – kinase (PI3K) enzyme a regulatory subunit of P85 α insulin receptor gene plays a major role in disposal of insulin-stimulated glucose [2]. Though mutations in P85α are rare in severe insulin resistance subjects, studies of PI3K enzyme in human syndromes of severe insulin resistance conducted in a cohert family, revealed three types of variants, due to mutations[3]. One type at codons 84 and the other at 221 are considered to be the silent polymorphisms as there are no amino acid changes, another at codon 326, a common variant showing no effect on the insulin stimulated PI3K activity and the third at codon 409 showing an impaired PI3K activity and suggests that it leads to insulin resistance. So the variant at codon 409 causing an amino acid change from Arginine to Glutamine had been attributed to the novel and real variant causing an impaired insulin stimulated PI3K activity leading on to the real insulin resistance. Thus, in this study, the structure of PI3K alpha and its binding mechanisms with the widely used anti-diabetic drugs which might pave a path to the close determination of significant variants of the PI3K alpha and its manifestation as insulin resistance were explored.

2. METHODOLOGY

Potential template

The protein sequence of PI3K alpha that plays a significant role in type II diabetes mellitus, was retrieved from the Gene database of Kyto Encyclopedia of Gene and Genomes (KEGG) [4]. The homologous sequences were obtained using NCBI-BlastP (basic local alignment search tool) by searching PDB [5]. The sequence with higher similarity was further selected as the potential template for homology modeling and the 3D structure of template was retrieved from the PDB [6].

Homology modeling of PI3K

The template and target sequence alignment file and the atomic coordinate file of the template structure were used as input to Modelere 9v9 to build the model. The 3D models are generated by satisfying the spatial restraints. A bundle of models was calculated and among the generated models, the the stereo chemical parameters were assessed by using SAVES server and the best model was selected for the further analysis [8].

Prediction of binding site

To find out the binding affinities between the modeled PI3K protein and 10 anti-diabetic drugs, the binding site residues of the developed PI3K model were predicted by using Q-site finder [9].

Ligand generation and flexible docking

The 2D structures of 10 anti-diabetics drug compounds were drawn in ACD-Chemsketch [10] and its SMILES notation was obtained. The SMILES notation used to converted into 3D SDF format at ‘Online SMILES convertor and Structure file generator’ [11]. The SDF structures were docked with the predicted binding site of PI3K alpha using Flex X [12] with default parameters as described[13]. The interactions of 10 anti-diabetic drug compounds with the amino acids within the binding site of PI3K alpha in the docked complex were analyzed by the pose-view of LeadIT.

3. RESULT AND DISCUSSION

Sequence search and Homology modelling

Using BLASTP, the PI3K alpha sequence was searched against PDB database and selected the crystal structure of P110 alpha (PDBID: 3HHM_A) as the best homologous sequences with E-value of 0.0. The PI3K alpha sequences and x-ray coordinates file 3HHM_A chain was used as inputs to build the model using Modeler9v9. The modelled structure of PI3K alpha is shown in Figure 1.

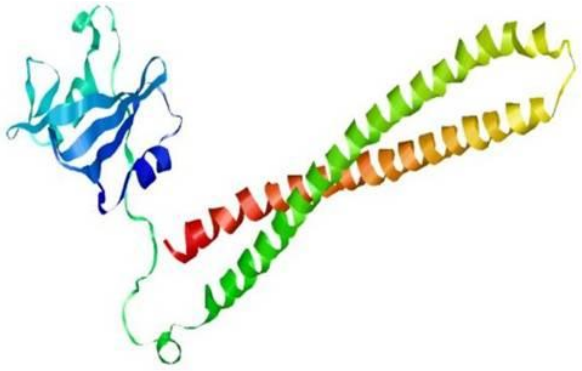

Figure 1: Modelled structure of PI3K protein with reference to PDBID 3HHM_A chain as template

Model Assessment

The generated models were further validated for their overall stereo chemical parameters by PROCHECK, Verfiy3D, and ERRAT of SAVES server. Using Ramachandran plot, the best models of PI3K alpha was considered for docking studies as it exhibited more number of residues in the most favorable regions and also the low number of residues in disallowed region. Figure.2a, b depicts the generated Ramachandran plots for both the modelled protein PI3K alpha and also its template 3HHM-A chain. The percentage of residues in the most flavoured regions, additionally allowed regions, generously allowed region and disallowed regions from modelled PI3K and its template 3HHM-A chain were given in the Table.1. Further the overall quality factor and compatibility of an atomic model (3D) with amino acid sequence (1D) for the modelled protein PI3K alpha and its template were also observed from ERRAT and Verify3D and tabulated. The results of Ramachandran plot, ERRAT and Verify-3D also confirms that the generated model was reliable and of good quality.

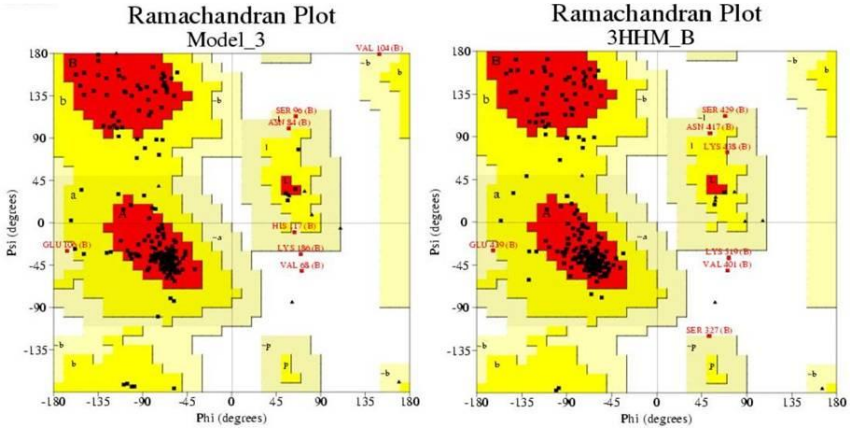

Figure 2 : (a) Ramachandran plot of the modelled structure (b) template 3HHM-B chain

Binding site Prediction

The modelled protein PI3K alpha was submitted to Q-Site finder and obtained TEN possible binding sites. Among the predicted binding sites, the first binding site (blue color) is considered as the

best binding site as ligand in the template structure is also observed in the same region and the same site is used for the further docking studies.

Table.1 Modeled Structure validation at SAVES server

Proteins	Number of Residues observed in Ramachandran plot and their percentage				G-factor	Verify 3D	ERRAT
	MFA	AAR	GAR	DAR			
Template (3HHM B chain)	198 (88.0%)	20 (8.9%)	5 (2.2%)	2 (0.9%)	-0.21	75.40%	89.573
Query	198 (85.7%)	26 (11.3%)	5 (2.2%)	2 (0.9%)	-0.14	74.90%	80.687

MFA-Most Favoured Region; AAR- Additionally allowed Regions ; GAR- Generously allowed Regions DAR- Disallowed Regions. G-factor- Goodness factor.

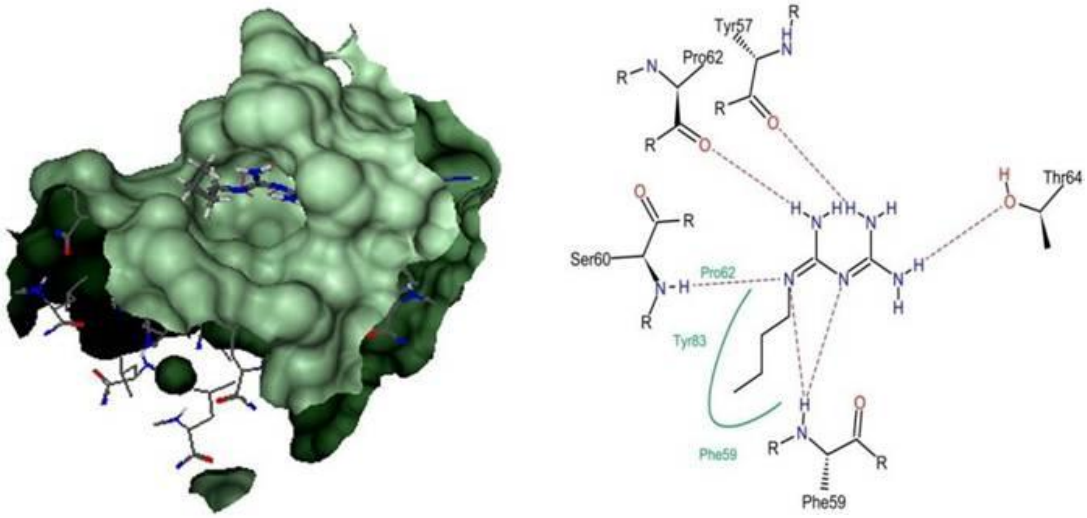

Figure 3a Docking complex and interactions of Buformin (-22.4387 kJ/mol)

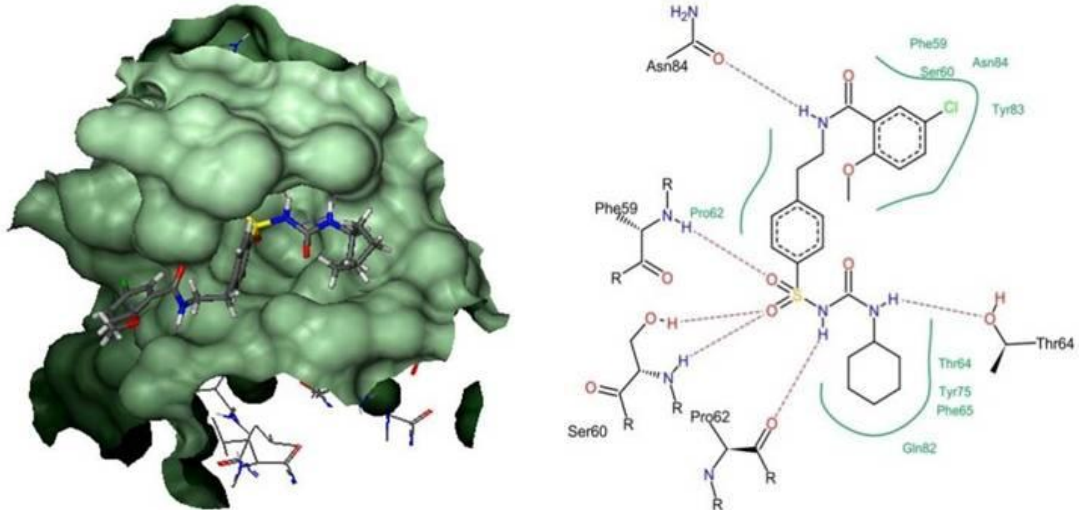

Figure 3b Docking complex and interactions of Glyburide (-22.7958 kJ/mol)

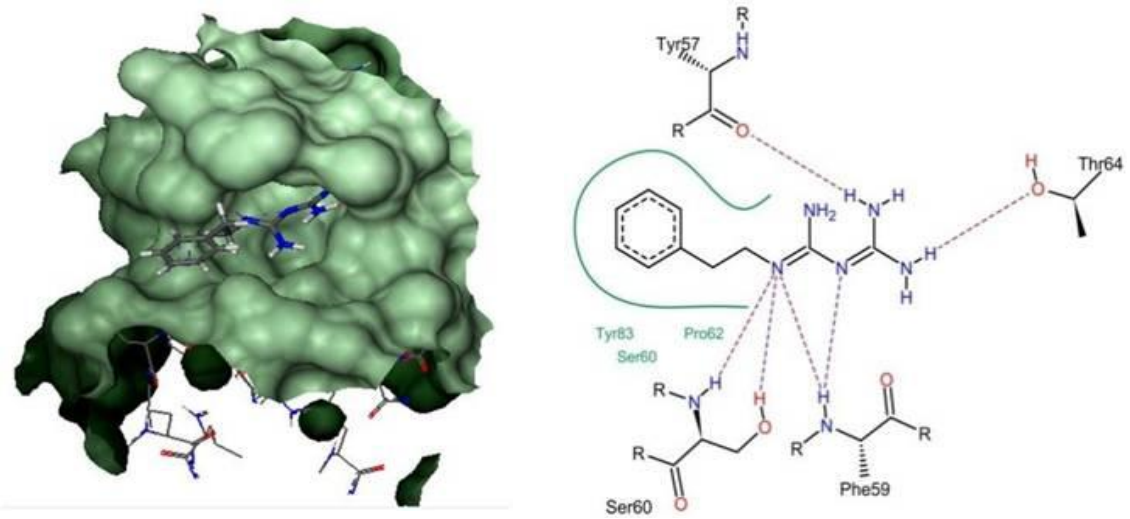

Figure 3c Docking complex and interactions of Phenformin (-24.1844 kJ/mol)

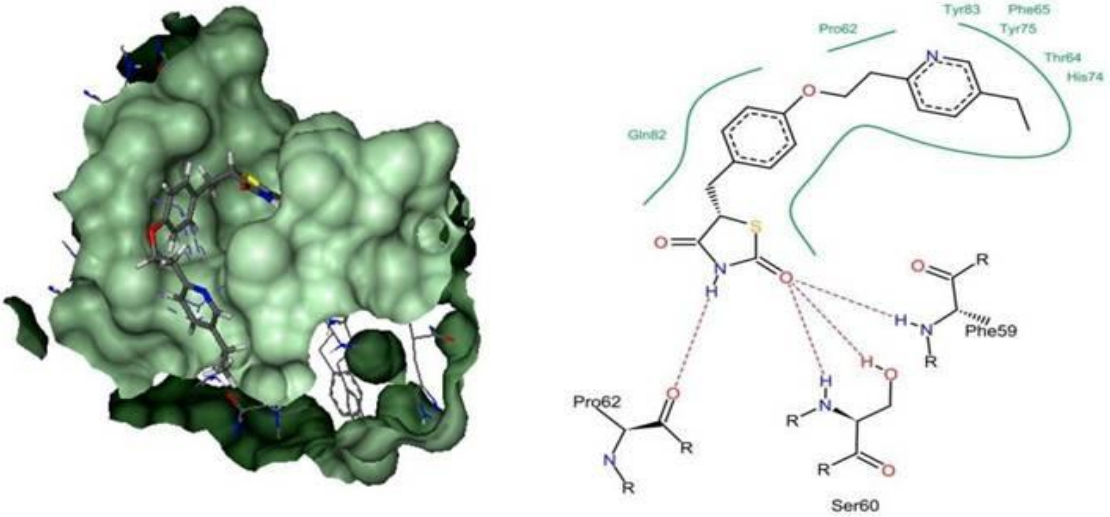

Figure 3d Docking complex and interactions of Pioglitazone (-21.3142 kJ/mol)

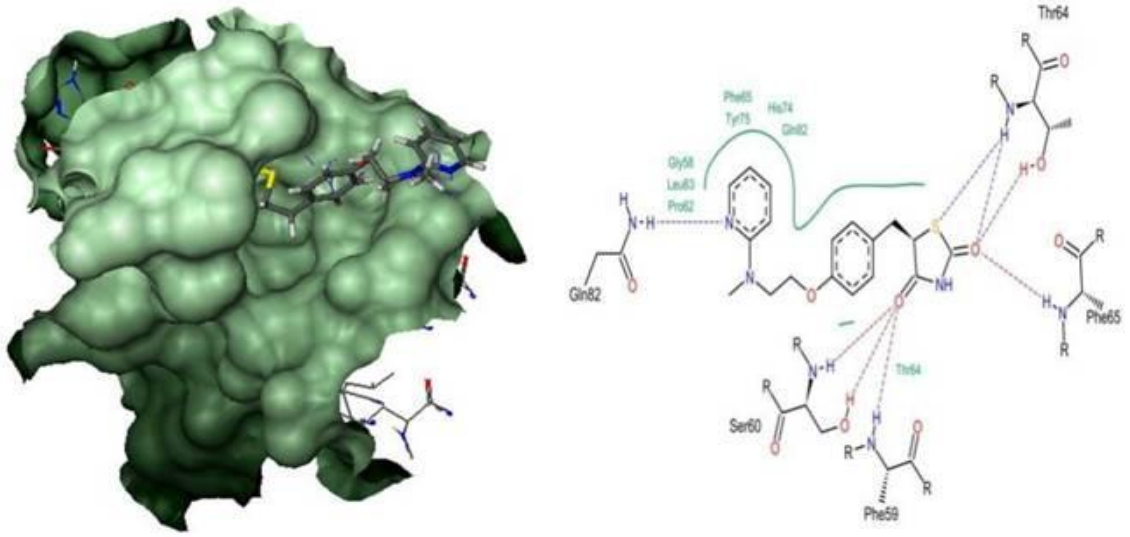

Figure 3e Docking complex and interactions of Rosiglitazone (-22.9938 kJ/mol)

Table.2 Docking interactions of TEN anti-diabetic drugs with their binding energies (kJ/mol)

S.No	Drugs	PubChemID	Interacting amino acids		Score (kJ/mol)
			Bonded	Non-bonded	
1	Metformin	CID-4091	Pro62, Ser60, Phe59, Tyr75	Tyr83, Phe59	-19.2387
2	Pioglitazone	CID-4829	Pro62, Ser60, Phe59	Gln82, Pro62, Tyr83, Phe65, Tyr75, Thr64, His74	-21.3142
3	Sitagliptin	CID-4369359	Ser60, Phe59	Leu63, thr64, gly58, Pro62, Phe65, His74, Gln82	-16.9710
4	Glyburide	CID-3488	Asn84, Phe59, Ser60, Pro62, Thr64	Phe59, Ser60, Asn84, Tyr83, thr64, Tyr75, Phe65, Gln82, Pro62	-22.7958
5	Repaglinide	CID-65981	Ser60, Phe59	Pro62, Thr64, Phe65, Gln82,His74	-15.0161
6	Rosiglitazone	CID-77999	Gln82, Ser60, Phe59, Phe65, thr64	Gly58, Leu63, Pro62, Phe65,Tyr75, His74, gln82, Thr64	-22.9938
7	Saxagliptin	CID-11243969	Thr64, Pro62, Ser60, Gln82, Phe59	Tyr83, gln82, Pro62, His74, Phe65, thr64	-13.9702
8	Phenformin	CID-8249	Tyr57, Thr64, Phe59, Ser60	Pro62, Tyr83, Ser60	-24.1844
9	Buformin	CID-2468	Thr 64, Phe59, Ser60, Pro62, Tyr57	Pro62, Tyr83, Phe59	-22.4387
10	Gliquidone	CID-91610	Tyr83, Asn84, Ser60, Phe59, Pro62	Ser60,Thr64,Asn84, Tyr83,Pro62,Gln82	-17.4837

Docking studies

Figure 3a-e show the docking complex and the interactions of 5 best antidiabetic compounds docked with in the binding site of modelled PI3K alpha. The docking interactions between the binding site residues of PI3K alpha (amino acids) and the 10 anti-diabetic compounds with their respective binding score were given in Table2. The docking interactions revealed that the Phenformin (CID-8249) is having strong interaction of - 24.1844 kJ/mol with PI3K alpha and the lowest interaction of -13.9702kJ/mol was observed with Saxagliptin. The favourable docking scores where due to the formation of Hbonds and also of hydrophobic interactions (Non bonded interactions). The docking studies of PI3K alpha with all the ten anti-diabetic compounds revealed that Phenyl alanine (Phe59) and Serine (Ser60) amino acids is mainly favoring the Hbond interactions. Whereas the Hydrophobic interactions (Non bonded) is flavoured by Tyrosine (Tyr 83) with most of the drugs. These findings suggested that the conserved Phenyl alanine (F), Serine (S) in the active site of PI3K alpha is crucial for anti-diabetic compounds binding. These docking interactions with the anti- diabetic compounds implies that the NH group and =O present in the anti-diabetic compounds favors the Hbond interactions in the active site of PI3K alpha. Hence it is suggested that, during the design of novel anti-diabetic compounds, the utmost care should be taken to consider the amino acids Phenylalanine, Serine and Tyrosine for posing a better interactions.

4. CONCLUSION

As the diabetes is attracting global importance, the studies on the real insulin resistance enzyme Phosphoinositide 3 – kinase (PI3K) enzyme, a regulatory subunit of P85 α insulin receptor gene that plays a major role in the mediation of insulin-stimulated glucose disposal is noteworthy. Hence in the present study the modeled structure of PI3K alpha and its binding studies with ten anti-diabetic compounds implied that Phenylalanine (Phe59), Serine (Ser60) and (Tyr 83) in the active site of PI3K alpha are essential for driving the interactions with anti-diabetic compounds. Thus the results of this study significantly shed light on the design of novel anti-diabetic compounds that can inhibit and overcome the real insulin resistance.

5. REFERENCES

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