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In-silico analysis of Porcine and recombinant Insulin activity on Glycogen

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Abstract

The study focuses on the anti-diabetic activity by molecular simulation of Recombinant Insulin, Porcine Insulin, and Glycogen. The sequence of these three molecules was retrieved, and 3D structures were modeled. A total of two different molecular simulations were carried out. The simulations were done using Autodock software. Initially, the downloaded PDB structures were docked with glycogen and the second between the active site peptide models of both insulin molecules based on castP prediction with glycogen molecule. The results were analyzed by Ramachandran plot for model prediction, and the binding energy was set as criteria to determine the best-docked model. The binding energy of recombinant insulin, porcine insulin with glycogen was 0.32 and -1.09 respectively. Similarly, the binding energy for peptide models with glycogen molecule was found to be +1.09 and +6.76 respectively. Based on the results, it was concluded that the recombinant insulin has higher affinity than the porcine insulin.

Keywords: Recombinant Insulin, Porcine Insulin, Glycogen, 7INS, 3i40, Molecular simulations, Active site prediction

1. Introduction

Insulin is a peptide hormone yielded by beta cells of the pancreatic islets; it is considered to be the optimal anabolic hormone of the body. It regulates the metabolism of carbohydrates, fats and protein by promoting the absorption of glucose from the blood [1]. Glycogen is multi-branched polysaccharide of glucose that serves as a form of energy storage in humans and animals. The polysaccharide structure represents the primary storage form of glucose in the body [2].

The abnormality in the production of insulin in the body leads to a state (or) disorder commonly known as Diabetes Mellitus. This usually occurs due to Pancreas's failure to produce insulin, Insulin Resistance (i.e.) Cells do not respond appropriately to insulin and Pregnant women with no prior record of the diabetes exhibit high blood sugar level [3].

Porcine insulin differs from human insulin by a single amino acid (Ala instead of Thr) at the C-terminal residue of the B-chain, a difference mostly invisible to the human immune system. Porcine insulin was

traditionally favoured by the Danish Insulin manufacturers since their farming industry was orientated towards Porcine rather than beef. Highly purified Porcine insulin is virtually indistinguishable from biosynthetic human insulin in its clinical effects, even though the latter is slightly more soluble and thus absorbed more rapidly [4]. Some patients have reported a loss of hypoglycemic warning symptoms on switching from Porcine to human insulin and should be treated with their preferred insulin, although evidence for this phenomenon is lacking. Though the Porcine insulin was effective yet still faces side effects such as rejection, instability etc. In order to overcome this recombinant insulin was developed.

Ever since the introduction of recombinant DNA technology, advances have been made in all sectors of biotechnology. rDNA technology was employed to construct a recombinant E.Coli expressing insulin. E.Coli is a preferred expression system for the proteins and other biopharmaceutical production. The recombinant insulin has been proven to be safe and effective in the treatment of diabetes. [5] [6].

Glycogen is a highly-branched polymer of around 30, 000 glucose residues and has a molecular weight between 106 and 107 daltons. Most of Glc units are attached by alpha-1, 4 glycosidic bonds, around 1 in 12 Glc residues makes -1, 6 glycosidic bond with a subsequent Glc which results in the creation of a branch [6][7]. Glycogen only has one reducing end and a large number of non-reducing ends with a free hydroxyl group at carbon 4.

Glycogen is in the form of granules in the cytosol in many cell types. When insulin levels in blood decrease, glycogen synthesis in the liver reduces and enzymes responsible for glycogen breakdown become active. Glycogen breakdown is stimulated by the presence of glucagon, which is secreted when blood glucose levels fall below the normal range [8].

Rational peptide design and large-scale prediction of peptide structure in sequence remain a challenge for chemical biologists. PEP-FOLD is an online service, focused on de novo modelling of 3D conformations for peptides between 9 and 25 amino acids in aqueous solution. Starting from an amino acid sequence, PEP-FOLD performs 50 simulations and returns the most representative conformations identified regarding energy and population [9].

Ramachandran plot is a way to visualize energetically allowed regions of backbone dihedral angles ψ against φ of amino acid residues in protein structure.

Molecular simulation is a method that predicts the preferred orientation of one molecule to a subsequent molecule when bound to each other to form a stable complex [10] [11]. Automated docking (Auto Dock) is used for the prediction of biomolecular complexes in structure/function examination and molecular design. Many methods are available, incorporating different trade-offs in molecular representation, energy evaluation, and conformational sampling to provide predictions with a computational effort [12][13]. AutoDock combines an empirical free energy force field with a Lamarckian Genetic Algorithm, providing a rapid prediction of bound conformations with analyzed free energies of association [14][15].

2. Methods

2.1 Sequence Retrieval:

The amino acid sequence for the required protein was obtained from UniProt (http://www.uniprot.org/). The accession no and the FASTA Sequence was obtained using UniProt server.

2.2 Structure Optimization:

2.2.1 Protein: Literature studies were carried out, and the structure was downloaded from NCBI_PDB server (https://www.rcsb.org/pdb/home/home.do). The Structure was optimized by removing the water molecules and the metal ion(Zn) using Auto Dock Software. Its molecular weight was analyzed using Chem Draw Software [16].

2.2.2 Ligand: The Glycogen structure was obtained from Pub Chem (https://pubchem.ncbi.nlm.nih.gov) server and was further optimized using Chem Draw Software. The structure was converted into PDB format using open babel software for molecular simulation [17] [18].

2.3 Active Site Prediction:

The active site of the protein was observed by using the CastP server (http://sts.bioe.uic.edu/castp/calculation.php) which provided the area, volume and the peptides which act as the binding site for the Ligand [19].

2.4 Peptide Model Synthesis:

The active site of the protein was examined using CastP server which was developed into a PeptideModel using PEP-FOLD server (http://mobyle.rpbs.univ-paris-diderot.fr) [20].

2.5 Ramachandran Plot Analysis:

The peptide models were studied using Ramachandran plot and the best model was selected for molecular simulation between the model and the Ligand.

2.6 Docking:

The docking process between the Protein and Ligand was carried out using the Autodock Software, and the results were analyzed by its binding energy. The docking process carried out in two sets. The first simulation was done between 7INS/3i40 molecule and glycogen. The second docking was carried out between the peptide models and glycogen molecule[21][22][23].

2.7 Molecular Visualization:

The results from autodock were visualized and were evaluated using Molecular Visualization Tools such as PYMOL and Chimera[24][25].

3. Results:

3.1 Sequence Retrieval:

The sequence for Porcine insulin was retrieved from UNIPROT with the accession no P01315 and for recombinant insulin the accession no was found to be P05019.Their respective FASTA sequence was also downloaded.

3.2 Structure Optimization:

The protein structures was optimized using Autodock software. The molecular weight of original protein was found to be 22180.5692 which was reduced to 20573.4861 after optimization. Similarly for recombinant it was 6546.45506 which was reduced to 5383.6965 after optimization (Fig1).The Ligand glycogen was optimized using Chem Draw software where its Bond length, Torsion angle and Energy were optimized (Fig 2).

3.3 Active Site Prediction:

The PDB file was uploaded in the CastP server where its pocket length and its binding sites were identified along with its peptides. The area of Porcine was found to be 240.5 and the volume was noted to be 282.2 and In case of Recombinant Insulin the area was observed to be 53.4 and the volume was 57.4. The peptide sequence details of porcine insulin (table 1) and recombinant insulin (table 2) are tabulated.

3.4 Peptide Model Synthesis:

The peptide sequence obtained from Cast P was uploaded in the PEP-FOLD Server which generated five models for porcine and three models for recombinant insulin. Upon further analysis the best model was selected for molecular simulation (Fig 3).

3.5 Ramachandran Plot Analysis :

The Ramachandran plot was used as a tool to predict the best model. Using Ramachandran plot the percentage of residues in each region such as favoured, allowed and outlier were calculated and the model with highest percentage of residues in favoured region was selected. The Ramachandran plot also gave us the complete sequence of the structure and the clefts available in the structure for docking purpose.

Porcine Insulin : The Five models were evaluated and the second model was found to have highest percentage of 83.9% in the favoured region and 16% in the outlier region (Fig 4).

Recombinant Insulin : The Three models were evaluated and the first model was found to have highest percentage of 100% in the favoured region and 0% in the outlier region (Fig 5).

3.6 Docking :

The Molecular Simulation was done in Autodock Software for both the set of proteins. The grid box was placed on the active site of the protein which was obtained from CastP server. The Search parameters were set at Genetic Algorithms and 50 runs were simulated. The results were generated based on the Lamarckian Genetic Algorithms. The results were tabulated (table 3).

Porcine Insulin: In the first simulation the original Porcine insulin PDB structure(7INS) was docked with glycogen the lowest binding energy of -1.09 was found in the 38^{th} run with the Mean Binding energy of +0.01. In the second simulation the peptide porcine model was docked with glycogen molecule, the least binding energy of +6.76 was found in the 4^{th} run with the Mean Binding energy of +27.78.

Recombinant Insulin: In the first simulation the original recombinant insulin PDB structure(3i40) was docked with glycogen the lowest binding energy of +0.32 was found in the 45^{th} run with the Mean Binding energy of +0.32. In the second simulation the peptide model of recombinant insulin was docked with glycogen molecule, the least binding energy of +1.09 was found in the 41^{st} run with the Mean Binding

energy of +4.91.

3.7 Molecular Visualization:

The docked Results were visulized using Chimera (Fig 6).

4. Conclusion

This study is mainly based on two types of insulin porcine insulin and recombinant insulin and their molecular interactions with glycogen molecule. The food we consume usually get stored in form of Glycogen in the body muscles, which on further interaction with insulin is converted into glucose which acts as a major source of energy to the body. Porcine insulin was previously used as major source of insulin production for a which is now replaced by Recombinant insulin expressed in ***E.Coli***. The molecular simulation (Docking) between Porcine Insulin/Recombinant Insulin with Glycogen will enable us to perform a comparative study between these interactions and help us to conclude the most efficient insulin among these two molecules.

The sequence was retrived based on the literature studies such as the acession no of **7INS** and **3i40** for Porcine and Recombinant insulin respectively. The glycogen molecule was obtained from PubChem server and was futher optimized using ChemDraw software. The molecular simulation was carried out using AutoDock software where the active sites were obtained from literature and was again confirmed using CastP server. The molecular simulation results were studied based on the binding energy. It gave us significant results, in case of porcine the least binding energy of -1.09 was found in 38^{th} run and for recombinant insulin it was 0.32 in the 45^{th} run. Based on this result it can be concluded that recombinant insulin has a better affinity than porcine since porcine insulin's binding score was too low.

PEP-FOLD: On retriving the active sites for both the insulin from CastP server, Peptide models were generated using PEP-FOLD server. Ramachandran plot was used to predict the best model on comparing the percentage of residues in the favoured regoin. Based on ramachandran plot results the prime porcine model had 83.9% residues in the favoured region and 0% residues in the outlier region. In case of Recombinant insulin, the ideal model had 100% in the favored region and 0% in the outlier region. These models were specified to be docked with glycogen molecule. The Molecular simulation results showed that the porcine had the least binding energy of 6.76 in the 4^{th} run and Recombinant insulin had the least binding energy of 1.09 in the 41^{st} run. On comparing these results, it was concluded that Recombinant insulin had better molecular interaction with glycogen as the binding energy of Porcine is too high.

The above results signified that the recombinant insulin has better binding affinity and molecular simulation with the glycogen molecule as its binding energy is moderate wheras the binding energy of porcine is either too high or two low. So its concluded that Recombinant insulin is better than Porcine insulin.

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5. References

1. Sonksen P, Sonksen J, "Insulin: understanding its action in health and disease," British Journal of Anaesthesia, vol 85, pp 69–79, (2000).

2. M Berger, S Hagg, N B Ruderman, “Glucose metabolism in perfused skeletal muscle. Interaction of insulin and exercise on glucose uptake”

3. Alberti, K.G.M.M. and Zimmet, P.Z, “Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus,” Provisional report of a WHO Consultation. Diabet. Med., 15: 539–553, (1998).

4. Ståhl F, Johansson R, “Diabetes mellitus modeling and shortterm prediction based on blood glucose measurements,” Math Biosci. Vol 217, pp 101-117, (2009).

5. Shivani Srivastava, Priya Shree, Harsh Pandey, Yamini Bhusan Tripathi, “Incretin hormones receptor signaling plays the key role in antidiabetic potential of PTY-2 against STZ-induced pancreatitis,” Biomedicine & Pharmacotherapy, vol 97, pp 330-338, (2017).

6. Tof I, "Recombinant DNA technology in the synthesis of human insulin". Little Tree Publishing, (2009).

7. Sonksen P, Sonksen J "Insulin: understanding its action in health and disease". British Journal of Anaesthesia, vol 85, pp 69–79, (2000).

8. Moses SW, Bashan N, Gutman A, "Glycogen metabolism in the normal red blood cell". Blood, vol 40, pp 836–43, (1972).

9. F. G. Young (1957). "Claude Bernard and the Discovery of Glycogen". British Medical Journal, vol 1, pp 1431.

10. B. Huang, “MetaPocket, a meta approach to improve protein ligand binding site prediction”, Omi. A.J. Integr. Biol. vol 13, pp 325–330, (2009).

11. D. Karthik, “Insilico docking analysis of dipeptidylpeptidase-4 (DPP-IV or CD26) with some selective bioflavonoids using genetic Lamarckian algorithm”, J. Comput. Methods Mol. Des, vol 4, pp 24–31, (2014).

12. Lengauer T, Rarey M, “Computational methods for biomolecular docking” Curr Opin Struct Biol, vol 6, pp 402–406, (1996).

13. Shoichet BK, Bodian DL, Kuntz ID, “Molecular docking using shape descriptors,”. J Comput Chem, vol 13, pp 380-397, (1992).

14. Schomburg D, Lessel U. Weinheim, “Lenhof H-P: An algorithm for the protein docking problem. In Bioinformatics: From Nucleic Acids and Proteins to Ceil Metabolism” pp 125-139, (1995).

15. VakserI A, “Protein docking for low-resolution structures,” Protein Eng, vol 8, pp 371-377, (1995).

16. Magni L, Raimondo DM, Bossi L, “Model predictive control of type 1 diabetes: an in silico trial,” J Diabetes Sci Technol, vol 1, pp 804-812, (2007).

17. Dalla Man C, Rizza RA, Cobelli C, “Meal simulation model of the glucose-insulin system,” IEEE Trans Biomed Eng, vol 54, pp 1740-1749, (2007).

18. Mohamed K. Ibrahim, Ibrahim H. Eissa, Mohamed S. Alesawy, Ahmed M. Metwaly , Mohamed M. Radwan, Mahmoud A. ElSohly, “Design, synthesis, molecular modeling and anti-hyperglycemic evaluation of quinazolin-4(3H)-one derivatives as potential PPARc and SUR agonists,”

19. Kool ET, "Active site tightness and substrate fit in DNA replication". Annual Review of Biochemistry, vol 71, pp 191–219, (1984).

20. Breton M, Kovatchev B, “Analysis, modeling, and simulation of the accuracy of continuous glucose sensors,” J Diabetes Sci Technol vol 2, pp 853-862, (2008).

21. Kroemer R, “Molecular Modelling Probes: Docking and Scoring” Portland Press Limited; (2003)

22. Vakser IA, Aflalo C, “Hydrophobic docking: a proposed enhancement to molecular recognition techniques,” Proteins, vol 20, pp 320-329, (1996).

23. Di Nola A, Roccatano D, Berendsen, “HJC: Molecular dynamics simulation of the docking of substrates to proteins,” Proteins, vol 19, pp 174-182 (1994).

24. Raval, A; Piana, S; Eastwood, MP; Dror, RO; Shaw, DE (August 2012). "Refinement of protein structure homology models via long, all-atom molecular dynamics simulations.". Proteins, vol 80, pp 2071, (2012).

25. Piana, S; Klepeis, JL; Shaw, DE, "Assessing the accuracy of physical models used in protein-folding simulations: quantitative evidence from long molecular dynamics simulations". Current Opinion in Structural Biology, vol 24, pp 98–105 (2014).

Figures:

Fig 1: Structure optimization of (a) porcine insulin and (b) recombinant insulin

(a) (b)

Fig 2: Structure optimization of glycogen molecule.

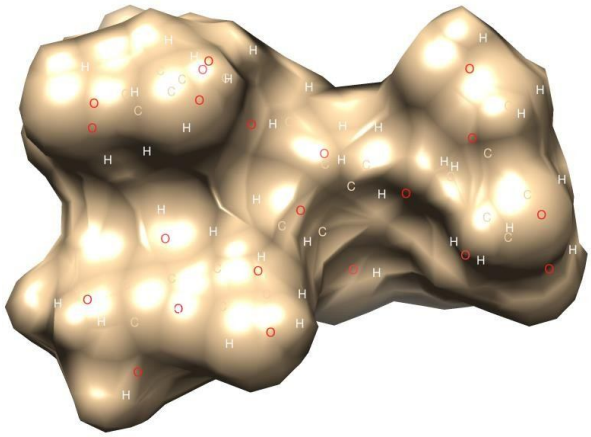
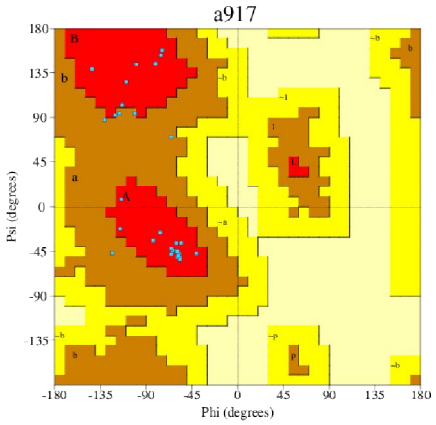


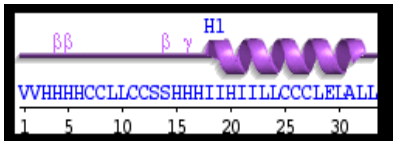
Fig 3: Peptide model synthesis of (a) porcine insulin and (b) recombinant insulin

(a) (b)

Fig 4: Ramachandran plot analysis of porcine insulin (a) Ramachandran plot (b) Peptide sequence (c) Porcine insulin clefts



(a)



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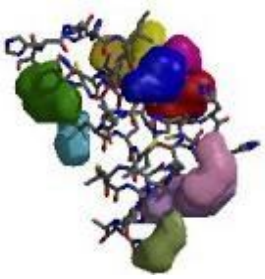
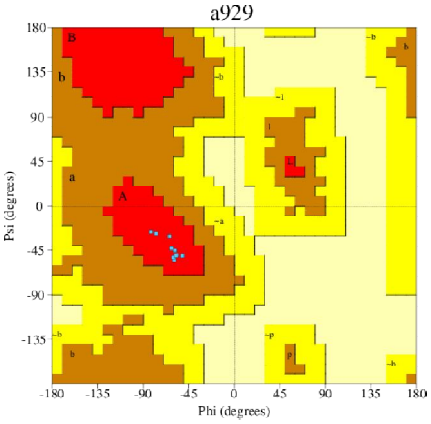
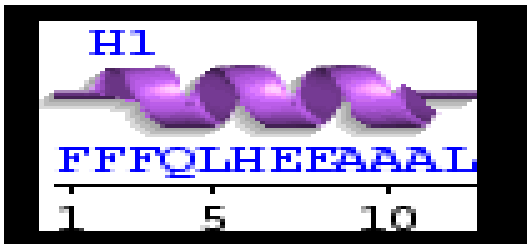


(c)

Fig 5: Ramachandran plot analysis of recombinant insulin (a) Ramachandran plot (b) Peptide sequence (c) Recombinant insulin clefts



(a)



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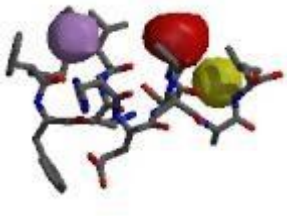
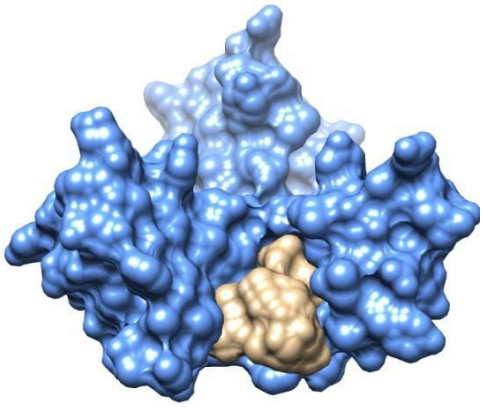
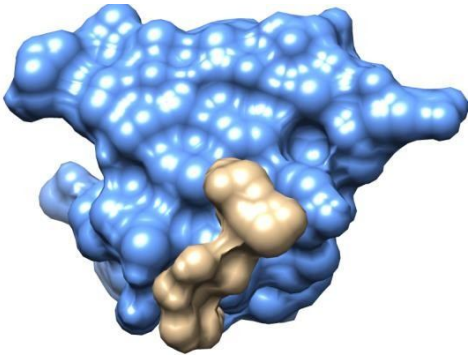


(c)

Fig 6 : Molecular visualization of (a) Porcine insulin docked with glycogen (b) Recombinant insulin docked with glycogen (c) PEP Fold model of porcine insulin docked with glycogen (d) PEP Fold model of recombinant insulin docked with glycogen



(a)



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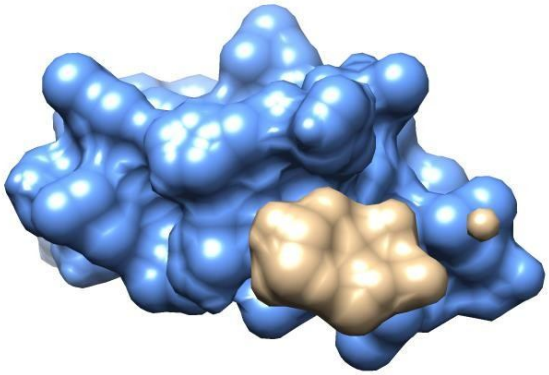

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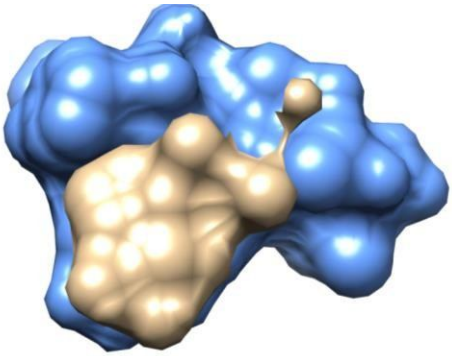

(d)

Tables:

Table 1: Active site prediction of porcine insulin

Positions	Peptide	Chain
2	VAL	F
2	VAL	F
5	HIS	F
5	HIS	F
5	HIS	F
5	HIS	F
6	CYS	C
6	CYS	C
6	LEU	F
6	LEU	F
7	CYS	D
7	CYS	D
9	SER	F
9	SER	F
10	HIS	D
10	HIS	D
10	HIS	D
10	ILE	C
10	ILE	C
10	HIS	D
10	ILE	C
10	ILE	C
11	LEU	D
11	LEU	D
11	CYS	C
11	CYS	C
11	CYS	C
13	LEU	C
13	GLU	D
13	LEU	C
14	ALA	D
16	LEU	C
16	LEU	C

Table 2: Active site prediction of recombinant insulin

Positions	Peptides	Chain
1	PHE	B
1	PHE	B
1	PHE	B
4	GLN	B
6	LEU	B
10	HIS	B
13	GLU	B
13	GLU	B
14	ALA	B
14	ALA	B
14	ALA	B
17	LEU	B

Table 3: Binding energy obtained from molecular simulations

S.No	Model	Binding Energy	Run
01	Porcine Insulin	-1.09	38
02	PEP FOLD Porcine Model	+6.76	4
03	Recombinant Insulin	+0.32	45
04	PEP FOLD Recombinant Model	+1.09	41