BIOINFORMATION
Discovery at the interface of physical and biological sciences

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Volume 8(22)

A docking study of insulin with LI-CR-L2 ecto domain of insulin receptor: an easy way for preliminary screening of novel anti-diabetic peptides

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Received September 24, 2012; Accepted September 27, 2012; Published November 13, 2012

Abstract:
Although interaction of human insulin with its receptor is studied to considerable extent such studies are currently lacking with recombinant insulin in-spite of its rampant clinical use. It is known that at molecular level the interaction of recombinant insulin with insulin receptor is similar to human insulin but not exactly same. With the increasing incidence of diabetes throughout the globe use of recombinant insulin is also increasing at a considerable rate. Therefore it is need of the hour to explore the recombinant insulin- insulin receptor interaction by all possible means. In this paper we have studied insulin receptor binding of lispro and glargine; the two commonly used recombinant insulins using tools of computational biology. We have observed that the binding pattern of insulin receptor (L1-CR-L2 ectodomain) with lispro and glargine is different when compared with human insulin. Analyzing the ligand receptor interactions we have hypothesized that the tail region of insulin beyond B26 is a critical regulator of insulin insulin receptor interactions detail of which cannot be understandable from docking studies due to lack of consideration of the flexibility of the tail region while docking studies. We have recommended experimental validation of our study. However, our docking procedure may also be explored for preliminary screening of novel anti-diabetic peptide.

Key Words: Diabetes mellitus, Glargine, Insulin, Insulin receptor, lispro.

Background:
Understanding receptor ligand interaction of insulin and its receptor has generated considerable current interest. At the present moment there are many studies that report both experimental and in-silico observations to document insulin insulin –receptor interactions **[1, 2].** Taking into consideration many limitations of such understanding theoretical modeling studies have been also published to develop a model of such interactions **[3].** Such studies are critical taking into consideration the wide spread emergence of diabetes mellitus and insulin resistance throughout the globe **[4].** To meet the need of the diabetes therapeutics that overcomes the limitations of human recombinant insulin therapy engineered insulins are currently in use **[5].** Clinical studies have proved that use of engineered insulins particularly insulin lispro and glargine are effective to control hyperglycemia and related complications and perhaps are better therapeutic alternative when compared with human recombinant insulin therapy **[5].** Although the therapeutic efficacy of insulin lispro and insulin glargine are considered to be proved and they are in regular clinical use but biological functions are not exactly identical to human insulin particularly when mutagenicity or receptor binding affinity of the above molecules are concerned **[6].** These may be important for practical point of view and already a group of studies claimed that insulin glargine use is associated with development of cancers although opposing view is also present

[7, 8]. Therefore it is need of the hour to study engineered insulin insulin receptor interactions which is lacking at the present moment. In-vitro studies have confirmed that L1-CR-L2 ectodomain of insulin receptor binds with insulin with considerable affinity **[9]** without participation of alpha CT region of insulin receptor which may be required for insulin binding to IR in-vivo (particularly relevant for insulin dimmers and hexamers) **[10].** But at the present moment there is complete absence of computational or experimental observation suggesting the mode of recombinant insulin interactions with L1-CR-L2 ectodomain of insulin receptor. Therefore, in this work we are reporting insulin receptor (L1-CR-L2 ectodomain), insulin lispro and insulin glargine interactions in comparison with human insulin using tools of computational biology.

Methodology:

The structure of human recombinant insulin, PDB code 1A7F is used for this study **[1].** To get the active conformation of human insulin and truncated insulin uptoB26 residue three mutations at positions B16, B24 and B30 in this structure have been modified using MODELLER **[11].** Further, the 3D structure of the insulin analogues – lispro and glargine have been modeled by using 1A7F as template structure in MODELLER. It is to be noted that in these cases also the mutations at positions B16, B24 and B30 have been considered. Insulin lispro is identical to human insulin but structurally contains just a transposition in the B28 and B29 amino acids. Insulin glargine has Glycine residue at position A21 of insulin instead of Asparagine and extra addition of two Arginine residues after B30 position. The ligand binding domain of insulin receptor (IR) is taken from PDB structure 2HR7 **[12].** The ligand receptor complexes of human recombinant insulin, truncated insulin upto B26, insulin lispro, and glargine have been built using docking tool (AUTODOCK 4.0) **[13].** The same is performed with mutated insulin receptor (F89L, F89W and F89A). In all these docking (both wild type and mutated receptor) the grid center is on x= 6.552, y=37.946 and z=58.854. F89L, F89W and F89A mutated insulin receptors have been constructed using MODELLER. PROFACE is used to calculate interface area, interface residues and interacting residues of ligand receptor complexes **[14].** Molecular diagrams are drawn by pymol **[15].**

Results:

Interface area of different insulin-insulin receptor complexes

Interface areas of human insulin, truncated insulin and recombinant insulin's with insulin receptor complexes both in wild type and mutated forms have been worked out and are given in **Table 1 (see supplementary material).** In case of insulin-insulin receptor complex, the interface area is 1507 Å^2 while in cases of recombinant insulins (lispro and glargine) the interface area is less, 1325 and 1449 Å^2, respectively. There is evidence that receptor binding affinity of lispro and glargine is less than normal human insulin **[6].** It is known that residues upto B26 of insulin are important for insulin receptor binding **[1, 16].** So we have truncated the wild type human insulin upto B26 residues and docked this truncated insulin with insulin receptor and F89L, F89W and F89A mutated insulin receptors. Truncated insulin-insulin receptor complex has 1835 Å^2 interface area while with mutated receptors interface area is less, 876, 1669 and 1551 Å^2, respectively. In case of truncated insulin-F89L mutated receptor the interface area is lowest. Mutated insulin receptor has less affinity towards insulin and F89 is a key residue of insulin receptor for insulin binding **[12].** We have mutated F89 by leucine, tryptophan and alanine and performed docking. The interface area is 1487 Å^2 between insulin-insulin receptor with F89L mutation. But in cases of tryptophan and alanine mutations the values are slightly higher, 1525 and 1685 Å^2, respectively. However, recombinant insulins – lispro and glargine have interaction with higher interface area with mutated insulin receptors in compared to wild type insulin receptor.

Interface and interacting residues of different complexes

Interface and interacting residues of insulin and truncated insulin with wild type insulin receptor are given in **Table 2 (see supplementary material).** The residues of insulin receptor which are present at the interfaces of both the complexes are mostly located on the β-sheet region of the ligand binding domain **(Figure 1a, b).** Using induced fit model Lou *et al*., 2006 have found that truncated insulin is bound with insulin receptor through this β-sheet region **[12].** We have also observed same result. The residues of insulin receptor that are known for binding with insulin are also common in our docking study. It is established that residues upto B25 of insulin B chain are required for insulin binding **[1, 16].** Both in normal and truncated insulins we have obtained similar results **(Table 2, Figure 1 a & b).** Lispro and glargine have also made complexes with insulin receptor in almost same orientation **(Table 2 & Figure 1e, f).** But, the residues located only on the middle strands of β-sheet have mainly interactions with lispro and glargine.

Discussion:

The receptor ligand interactions of insulin and insulin receptors are experimentally worked out [**1, 2, 9, 12, 17]**. But that is not the case with insulin lispro and glargine. Keeping in view the widespread use of these two engineered insulin in diabetic individuals our observed results are important. From experimental studies, the critical moieties of insulin insulin receptor interactions are known **[1, 2, 12].** When we have docked B26 truncated insulin with insulin receptor the interaction happened through the experimentally known β strand region with the majority of the amino acid interactions are same as observed in experimental study **(Table 2 & Figure 1b) [12].** When we have docked the insulin with its receptor similar results are observed **(Table 2 & Figure 1a).** But when we have docked B26 truncated insulin with F89L or F89A or F89W mutated insulin receptor the binding is observed to be drastically reduced **(Table 1 & Figure 1d)** and similar phenomenon is observed when insulin is docked with F89L mutated receptor **(Table 1 & Figure. 1c).** Since F89 of insulin receptor is experimentally proved to be critical for insulin binding and various mutations of F89 are known to reduce the insulin binding very significantly **[12]** we are in the opinion that our above mentioned observed results are in conformity with the experimental results and the mode of docking studies we have relied upon can give us realistic information. In case of F89W and F89A of insulin receptor mutations docking results with insulin have produced unrealistic results **(Table 1)** which are not in accordance with the experimental observations **[12].** Therefore from docking studies with insulin molecule we should not rely on the results obtained with F89W or F89A mutated receptor. There is possibility that since alanine is a small residue and the hydrophobicity of phenylalanine and

tryptophan is similar the docking results are producing unrealistic results. Therefore the tail of the insulin has the potential to produce unrealistic docking results with insulin receptor which is not apparent in B26 truncated insulin. There is evidence that the tail of insulin has flexibility while receptor binding **[1, 2]** which the docking procedure is also not taking into account and can be counted as an explanation for such unrealistic docking results.

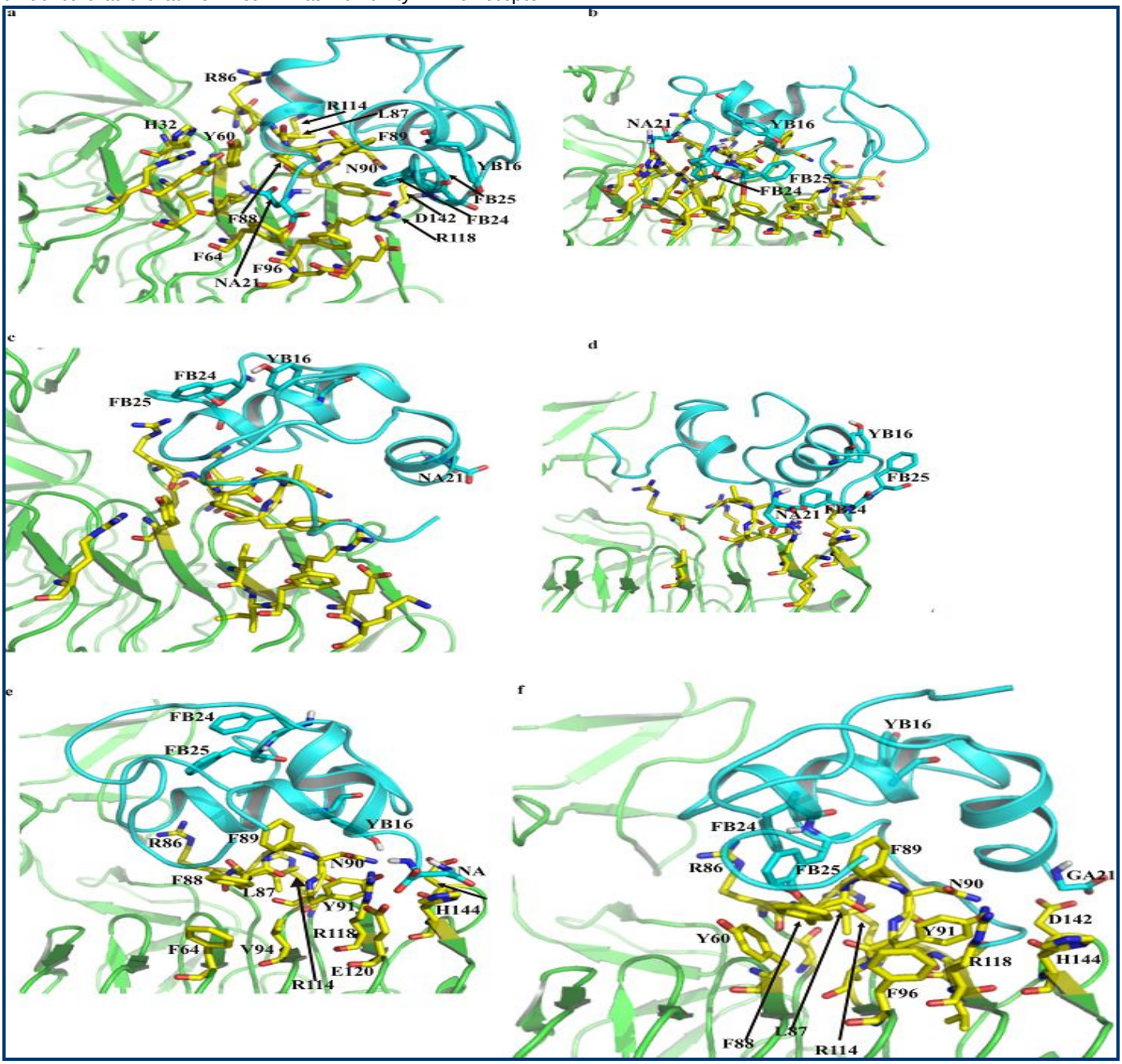

Figure 1: Ligand –receptor interactions between **a)** insulin-insulin receptor; **b)** truncated insulin-insulin receptor; **c)** insulin-F89L mutated insulin receptor and **d)** truncated insulin-F89L mutated insulin receptor; **e)** lispro-insulin receptor and **f)** glargine-insulin receptor are shown. The ligand binding region of insulin receptor is represented in green cartoon while insulin, lispro and glargine are represented in cyan cartoon. In **Figure 1a,** the residues of insulin receptor which are important for insulin binding are shown in stick mode with carbon, nitrogen and oxygen atoms are in yellow, blue and red color, respectively. Four residues of insulin / lispro / glargine – AsnA21 / GlyA21 (in case of glargine), TyrB16, PheB24 and PheB25 are shown in stick mode with carbon, nitrogen and oxygen atoms in cyan, blue and red color, respectively. In **Figure 1e & 1f**, the residues of insulin receptor which are involved in binding with lispro and glargine are shown in stick mode with carbon, nitrogen and oxygen atoms are in yellow, blue and red color, respectively. All residues are labeled by one-letter amino acid code and residue number with chain id in case of insulin / lispro / glargine only.

In case of insulin lispro and insulin glargine docking with insulin receptor, it is showing interactions with residues of the middle part of β sheet of insulin receptor and this is making the receptor ligand interactions with a smaller binding area **(Table**

1,2 & Figure 1). It is known from experimental studies that activity of lispro and glargine is less when compared to human insulin **[6].** Taking these facts into consideration we feel that the observed docking results has realistic implications although experimental validation of the observed results in this connection is lacking at the present moment. We feel that since these two molecules are approved drug to treat diabetes mellitus, urgent experimental validation of the docking result is important. Even with F89L insulin receptor mutation docking with lispro and glargine is producing receptor ligand complex with more binding area **(Table 1).** Experimental validation is lacking at the present moment for such observation which should be done taking into consideration widespread use of lispro and glargine in diabetic subjects. Although lispro and glargine produces physiologically comparable functions our body does not produce it. The interface area and interacting residues of insulin and insulin receptor which regulate binding energy are important for proper function and binding energy will be changed in case of glargine and lispro. However, experimental validation of these results are lacking at the present moment and requires urgent validation particularly with reference of carcinogenicity of glargine as claimed in some studies. The difference in interaction pattern of lispro and glargine with insulin receptor is due to altered mode of binding mainly due to change in the amino acid residues beyond B26. Therefore we are in opinion that the tail region of insulin beyond B26 is a critical regulator of insulin insulin receptor interactions detail of which cannot be understandable from docking studies due to lack of consideration of the flexibility of the tail region while docking studies. We advocate urgent validation of lispro and glargine interaction with insulin receptor keeping the observations of this study into consideration.

So far we have discussed the results of the docking studies of insulin receptor (L1-CR-L2) ectodomain with insulin and its analogues based on the previous experimental observations **[12].** But a question is unanswered - will the findings of docking results be exactly simulated in-vivo state? The answer in one word is No since now it is established that A2, A3, A4, A8, A14, B25, and B27 residues of insulin interact with the alpha CT segment of insulin receptor **[18]** some of which are observed to be interacting with the insulin receptor in our study **(Table 2).** This result is obtained due to the fact that we have not docked an alpha CT interacted insulin molecule with insulin receptor because alpha CT binding is not absolutely necessary for insulin binding to its receptor. In fact in vitro studies with expressed L1-CR-L2 ectodomain of insulin receptor had shown binding with insulin without even presence of alpha CT region **[12, 19].** The previous experimental studies have done binding studies of insulin with only L1-CR-L2 ectodomain of insulin receptor. The basic objective of this paper is to predict the interactions of recombinant insulins with the L1-CR-L2 ectodomain of insulin receptor so that analogous experiments can be framed keeping previous experimental design in mind for the purpose of screening of novel peptide binding with insulin receptor. We think that our observations demonstrate result in that direction.

Acknowledgement:
RB and DB acknowledge DST, New Delhi for financial assistance for the work.

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Edited by P Kangueane
Citation: Bhattacharyya & Banerjee, Bioinformation 8(22): 1082-1086 (2012)

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Supplementary material:

Table 1: Interface area of different insulin and insulin receptor complexes are given.

Ligand	Receptor	Interface area (Å^2)
Insulin	Insulin receptor	1507
Insulin-B26	Insulin receptor	1835
Lispro	Insulin receptor	1325
Glargine	Insulin receptor	1449
Insulin	Insulin receptor-F89L	1487
Insulin-B26	Insulin receptor-F89L	876
Lispro	Insulin receptor-F89L	1873
Glargine	Insulin receptor-F89L	2127
Insulin	Insulin receptor-F89W	1525
Insulin-B26	Insulin receptor-F89W	1669
Lispro	Insulin receptor-F89W	1837
Glargine	Insulin receptor-F89W	1859
Insulin	Insulin receptor-F89A	1685
Insulin-B26	Insulin receptor-F89A	1551
Lispro	Insulin receptor-F89A	1454
Glargine	Insulin receptor-F89A	1500

Table 2: Interface and interacting residues of insulin and insulin-B26 with insulin receptor is given below. The bold residues of insulin receptor are common in both complexes. The residues of insulin receptor which are common in interaction with lispro and glargine are in italics. In Glargine this residue is glycine.

Residues of insulin receptor		**Residues of ligand**	
In interface of the complex	**Interacting with ligand**	**In interface of the complex**	**Interacting with insulin receptor**
	Insulin receptor and insulin complex		
R14, H32, Q34, L36, L37, Y60, L62, F64, S85, R86, L87, F88, F89, N90, Y91, Y94, F96, R114, G115, S116, R118, E120, K121, D142, H144, G273, H275	**H32**, Q34, L36, **Y60**, L62, **F64**, S85, ***R86***, ***L87***, ***F88***, ***F89***, ***N90***, Y91, **F96**, E97, ***R114***, ***R118***, E120, ***D142***	I2A, V3A, E4A, Q5A, C11A, S12A, Y14A, Q15A, N18A, Y19A, C20A, N21A, L11B, A14B, L15B, R22B, G23B, F24B, F25B	I2A, V3A, E4A, ***Q5A***, C6A, Y14A, Q15A, ***L16A***, ***N18A***, ***Y19A***, ***C20A***, ***N21A****, ***L11B***, ***L15B***, ***R22B***, G23B, F24B, ***F25B***
	Insulin receptor and insulin-B26 complex		
G10, D12, R14, G31, L37, H32, Q34, L36, D59,Y60, L62, F64, R86, L87, F88, F89, N90, Y91, F96, R114, R118, E120, K121, E141, D142, N143, H144	D12, R14, **H32**, Q34, L36, L37, D59, **Y60**, L62, **F64**, **R86**, **L87**, **F88**, **F89**, **N90**, **F96**, **R114**, **R118**, E120, E141, **D142**, N143, H144	I2A, V3A, E4A, Q5A, C7A, Q15A, L16A, E17A, N18A, Y19A, C20A, N21A, H5B, C7B, G8B, L11B, V12B, L15B, A14B, R22B, G23B, F24B, F25B	E4A, **Q5A,** C7A, Q15A, L16A, E17A, N18A, Y19A, C20A, N21A, H5B, C7B, G8B, L11B, V12B, L15B, A14B, R22B, G23B, F24B, F25B