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3D Structure of Resistin, A Molecule Responsible for Insulin Resistance: An Computational Approach

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Resistin, a unique signaling molecule involved in type 2 diabetes. It is secreted by adipose tissue. The human resistin consists of 108 amino acids. Protoparam and protascale servers are used to access various general properties of resistin. Modeler, a well known homology modeling tool is used to model the tertiary structure of resistin. Quality of the structural models are evaluated by using procheck and verify 3D and the best model is optimized by swissPDB viewer. PSORT and SignalP servers are used to determine localization and signal peptide of resistin, respectively. It is predicted that resistin starts with highly hydrophobic amino acids and has conserved pattern of cysteines at the C-teminal like any other members of FIZZ family. It consistis of both α-helix and β-sheet. It is hypothesized that resistin has N terminal signal sequence with highly hydrophobic amino acids and is localized extracellularly. Hence it may be exerting its action through a surface receptor, which may negatively regulate insulin signaling pathway and cause dibetes.

Key Words: Adipocyte, Resistin, Insulin, Type 2 diabetes.

INTRODUCTION

Earlier type 2 diabetes mellitus was considered as a rare disease but recently there has been a tremendous increase in the incidence of type 2 diabetes. About 16 million Americans have type 2 diabetes[1]. Obesity is associated with insulin resistance and is the biggest risk factor for type 2 diabetes. The hormone responsible is reported to be resistin, which is secreted by adipocytes during its differentiation. It prompts the tissue to resist insulin and it is believed that resistin may form at least part of the missing link between obesity and diabetes[2]. Mice given resistin were not able to process blood sugar and mice given a drug that lowers resistin levels were better able to process blood sugar by using insulin. A new class of antidiabetic drugs, thiazolidinediones (TZDs) are known to reduce insulin resistance and this has been hypothesized that it lowers adipocyte differentiation and in turn lowers the release

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of resistin[3]. However, the molecular mechanism of resistin is not known. The tertiary structure of the protein required to be known to understand the molecular mechanism of resistin. Since the experimentally solved structure is not known, an attempt is made to model its structure. The 3D model of resistin may help to develop effective drugs which can block resistin activity selectively.

EXPERIMENTAL

The general properties of resistin are predicted from protoparam and protascale (http://www.expasy.ch). Various paramerters like hydrophobicity[4] bulkiness[5], percentage of accessible residues[6], average buried area and average flexibility[7] are assessed. Average area buried gives the measure of hydrophobicity. Resistin is identical to members of FIZZ family[8]. Resistin has N-terminal signal peptide and unique conserved pattern of C-terminal cysteines (X11-C-X8-C-X-C-X3-C-X10-C-X-C-X-C-X9-CC-X3-6-END) which are identical to other members of FIZZ family.

The primary sequence of resistin was submitted to SignalP server[9] for the prediction of signal peptide and it was predicted that the signal peptide starts form amino acid no: 1 to 19. Protein localization server PSORT[10] predicted resistin localization. Tertiary structure of resistin is modeled using the following methods: (1) Swiss Model[11]; (2) Homology Modeling (http://www.cmbi.kun.nl/swift/future/aainfo/)

Swiss model: It is an online modeling tool models automatically the 3D structure of protein using homology modeling. It could not model the 3D structure since there is no identical template structure available for resistin.

Homology modeling: The PDB data base was searched to obtain a structural template for Homology modeling using BLAST[12]. Most of the sequences obtained from the Blast search were having significant homology with the resistin sequence. But none of the sequences have known structure in PDB database. Hence an attempt was made to obtain a distant homologue using PSI Blast 1. The obtained sequences had significant homology but no sequence had a known structure in PDB.

Threading: An attempt is made to get the template from SCOP[11] database. The secondary structure is predicted using the PHD[13] PredictProtein (http://www.embl-heidelberg.de/predictprotein/predictprotein.htm), GOR4[14]. Both the tools consensually predicted that the given sequence possessed α- and β-strands. By using the secondary structure information a search was made through the hierarchical classification of SCOP[15]. But this search is not able to predict a suitable template, since resistin does not belong to any structural family.

LOOPP: The resistin sequence was searched against LOOPP (http://www.tc.cornell.edu/reports/NIH/resource/CompBiol/Tools/loopp) database. The best matching sequence was 2ALR and it was considered as the structural template to model the structure of resistin.

Modeling: Modeller[16] was used to model the structure.

Model refinement: Among the ten models obtained one model was selected based on the least objective function value. The same model was subjected to model

refinement by Swiss PDB Viewer[11] and the quality of the model was evaluated using verify 3D (http://www.doe-mbi.ucla.edu/Services/Verify_3-+\.D/) and procheck[17].

RESULTS AND DISCUSSION

In the present study, Homology modeling was used to model resistin's structure. It is well known that Homology modeling is a successful technique for protein modeling at CASP (http://predictioncenter.llnl.gov/). Protoparam analysis predicted that this protein starts with a stretch of amino acids with high hydrophobic values and ends with a stretch of amino acids with low hydrophobic values. It has 9 negatively charged residues (Asp+Glu) and 9 positively charged residues (Arg+Lys). The GRAVY (Grand average of hydropathy) score of resistin is 0.381 according to protoparam analysis. This score is very close to GRAVY score[18] of human glucose carrier *i.e.* 0.37, a membrane spanning protein. The protein localization server PSORT predicted that the resistin might localize extracellularly. Bulkiness was predicted using protascale tool, which shows that a stretch of bulkier residues is present at the beginning of the protein (1-20) with intermittent stretches of bulkier residues (Fig. 1B).These bulkier residues may be well suited to occupy the interior of the protein. This agrees well with the pattern for % of accessible residues (Fig. 1C) The pattern of average area buried (Fig. 1E) is observed to be complementary not only to pattern of average flexibility (Fig. 1D) but also to pattern of % of accessible residues (Fig. 1C). Hence it indicates that the residues accessible to solvent may be present at flexible areas like loops and turns.

Signal P results predicted that there is an N-terminal signal sequence cleaving at residue no. 19. Almost all proteins that will be secreted extra-cellularly are initially delivered to the endoplasmic reticulum[19] and proteins destined for initial transfer to endoplasmic reticulum possess a signal peptide at its N-terminus, characteristically including a sequence of hydrophobic amino acids[20]. It is observed in Fig. 1A that up to residue no. 19 all the residues have high hydrophobic values. All the above obser-vations led to make following hypothesis.

Resistin may initially be delivered into endoplasmic reticulum lumen. The protein that is delivered into endoplasmic reticulum lumen will finally be translocated to the exterior of the cell as predicted by PSORT, a protein localization server that resistin is localized extracellularly. During the translocation of the protein through the endoplasmic reticulum membrane, the N-terminal signal peptide may be cleaved off.

Modelling: The best matching sequences for resistin sequence from LOOPP database is 2ALR and LOOPP search results are shown in Table-1.

The alignment of resistin with best matching sequence-2ALR is given below:

query= Resistin match= 2alr energy= -96.00 length= 86 type= local(seq-seq)

KALCLLLLPVLGLLVSSKTLCSM-

EAINERIQEVAGSLIFRAISSIGLECQSVTSRGD 2-59 query

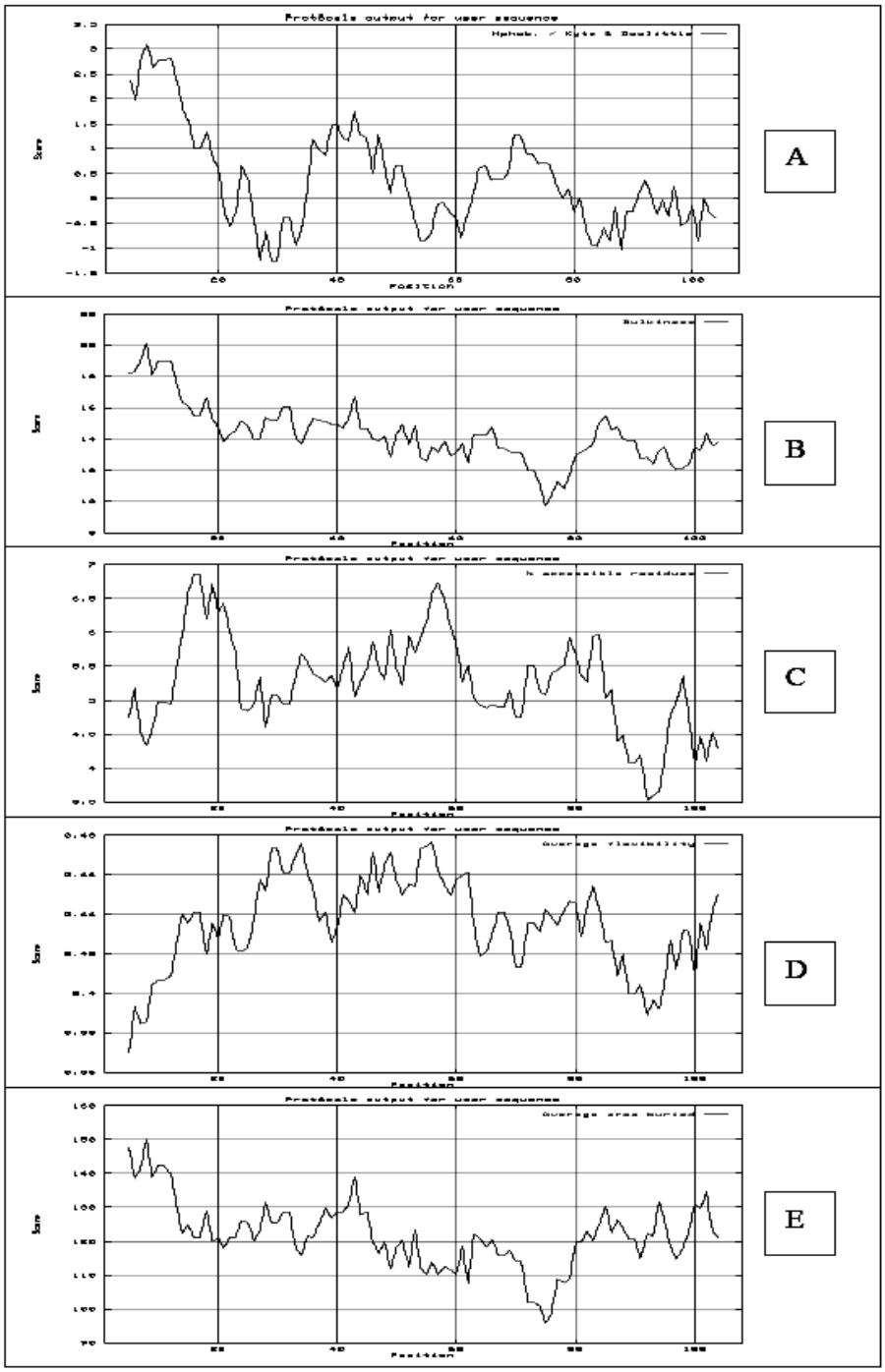

Fig. 1A: Hydrophobicity, Fig. 1B: Bulkiness, Fig. 1C: % of accessible residues, Fig. 1D: Average flexibility, Fig. 1E: Average area buried

TABLE-1
RESULTS OF LOOPP SEARCH

PDB-ID	Energy	Z-score	Significance
2alr	-96.0	5.1	High
1gpl_A	-96.0	4.3	High/Low
2axe	-95.5	4.1	High/Low
1hre	-97.5	3.7	High/Low
2alp	-101.5	3.4	High/Low
1ah1	-101.0	3.4	High/Low
1lsi	-113.5	3.3	High/Low
4gsa_A	-89.5	3.0	High/Low
3cd4	-84.0	3.0	Low
1hgeA	-87.5	2.9	Low
1aun	-104.5	2.8	Low
2ncd_A	-89.5	2.5	Low
1mfs	-85.5	2.5	Low
1onc	-85.5	2.3	Low
11bh_A	-91.0	2.2	Low

KALEALVAK--G-LVQALGLSNFNSRQIDD-ILSVA-
S-V-R-AVLQVECHPYLAQNE 144-194 match
L-ATC-PRGFAVTGCT-CGSACGSW-D 60 - 82 query %align= 75.0 %ident= 33.3)
LIAHCQARGLEVTAYSPLGSSDRAWRD 195 - 221 match (%align= 24.1)

The sequence was retrieved from PDB by using the PDB id:2ALR. From the PDB file it was observed that 2ALR is an aldehyde reductase enzyme involved in diabetic complications. So 2ALR had some functional similarity with resistin In addition to this, 2ALR had high significance and high Z-score (Table-1). From the above observations it was concluded that 2ALR is the best structural template for modeling the structure of Resistin. All the required files were submitted to modeler for homology modeling of resistin 3Dstructure. The modeller returned 10 putative models of resistin. Out of the 10 models, the one with low object function value was selected for further model refinement. The model was refined by Swiss PDB viewer and verified by Verify-3D.

According to verify-3D results shown graphically in Fig. 2, 1 to 24 residues are having negative 3D profile values and the remaining positive. It was not possible to refine the quality of that structural segment (1 to 24). But it was found that the original sequence of resistin starts from residue[18]. The sequence upto 19th residue is predicted to be the signal sequence.

The stereo chemical quality of the refined model was checked by Procheck and the results were as follows:

Plot statistics: Residues in most favoured regions: 86.2 %, Residues in additional allowed regions: 12.8 %, Residues in generously allowed regions: 1.1 %, Residues in disallowed regions: 0.0 %, The structural model of Resistin was submitted to PDB, the PDB id is 1LV6 and its structure is shown in Fig. 3.

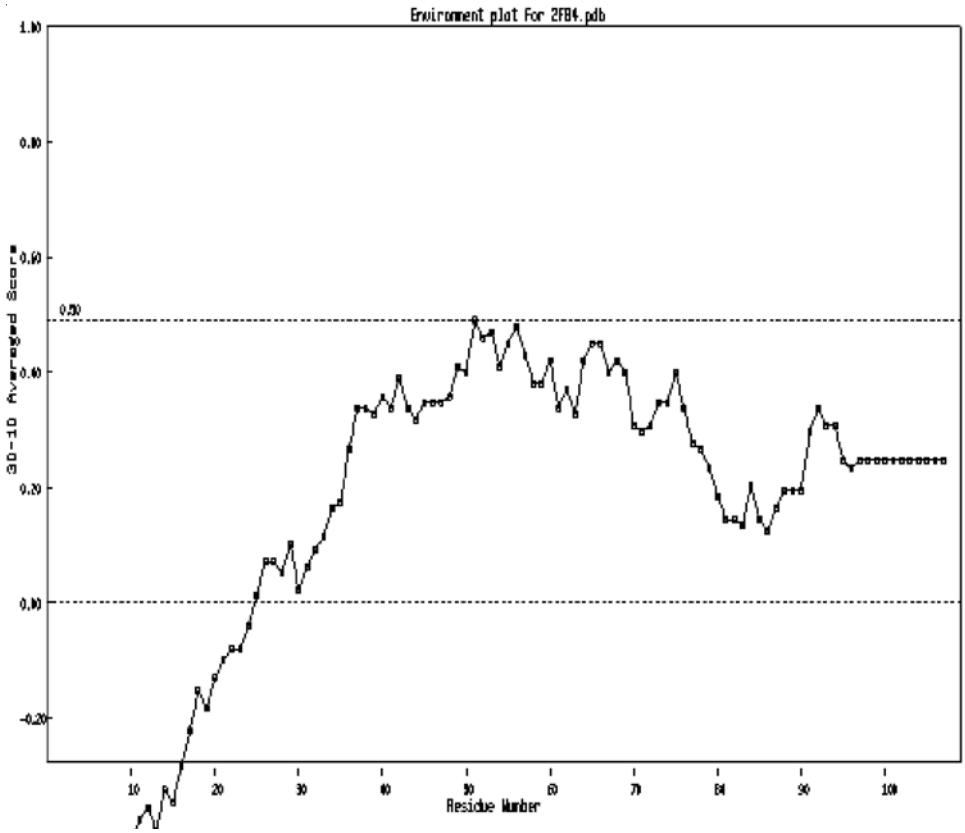

Fig. 2. Graphical representation of SwissPDB viewer results

Fig. 3. Putative structure of resistin

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