Volume: I: Issue-3: Nov-Dec -2010 IJABPT ISSN 0976-4550

Research Article

HOMOLOGY MODELING AND ACTIVE SITE ANALYSIS OF LEPTIN RECEPTOR OF *HOMO SAPIENS* INVOLVED IN OBESITY

Chauhan Poornima[2], Siddiqui M. Asif[1*], Amir Asad[1], Goyal Vibhika[3], Singh Jitendra[4], Singh Raghvendra[4]

[1]Dept. of Biotechnology, M.I.E.T., Meerut.

[2]Dept. of Bioinformatics, C.A.E.H.S., Meerut

[3]Dept. of Microbiology, C.A.E.H.S., Meerut

[4]Dept. of Biotechnology, S.V.B.P.T.U. of Ag. & Tech., Meerut

*Corresponding author email: - asifsiddiqui82@gmail.com

Phone: - +91-9410606339

ABSTRACT: The discovery of leptin has initiated a flurry of research into the molecular basis of weight control. In obese people levels of leptin found in the blood are normally very high and more than sufficient to suppress the appetite and increase the metabolism. This however does not happen and it is believed that obesity may be the result of a resistance to leptin. This suggests that the problem in these individuals may be related to a lack of binding of the leptin protein to its receptor. No known structure of leptin receptor is known. Therefore in present the present study we model the 3D structure of leptin receptor using MODELLER. This was done using the template GP130 of *H. sapiens* (PDB code: 1BQU). On the basic of results MODEL 6 was selected as the best model. The observed G-factors for the present model were -0.22 for dihedrals, -0.32 for covalent and overall -0.25. The MODEL 6 contains 88.7% of the residues in the most favored region, 11.3 % in the additional allowed and no single residue in generously allowed regions and disallowed region. The predicted model was further analyzed to locate the residues in the active sites those provide interactions with the ligand.

Keywords: BLASTP, Homology modeling, Leptin, PDB

INTRODUCTION

Obesity, a condition of excessive fat accumulation in adipose tissue**,** assessed by using body mass index (Calle *et al*., 1999). Today, Obesity continues to be a major health concern all over the world but it has also begun to be ranked as a serious risk comparable to certain diseases (Allison *et al*., 1999). Obesity has reached epidemic proportions globally, with more than 1 billion adults overweight out of which at least 300 million are clinically obese and pose a major risk for serious diet-related chronic diseases, including type 2 diabetes, cardiovascular disease, hypertension, stroke, and certain forms of cancer (Barness *et al*., 2007).

Several factors may contribute to the development of obesity. Many proteins are involved, among them leptin plays center role in regulating energy intake and energy expenditure, including appetite and metabolism (Barness *et al*., 2007). Once leptin has bound to the leptin receptor, it activates the stat3, which is phosphorylated and travels to the nucleus to, presumably, effect changes in gene expression. The appetite is controlled by two types of neurons in the hypothalamus of the brain are NPY/AgRP and POMC/CART (Banks *et al*., 2004).

Once leptin has bound to the Ob-Rb receptor, it activates the stat3 signal via the JAK/STAT pathway (GreGreen *et al*., 1995). In the NPY/AgRP neurons. Further, leptin suppresses the expression of NPY and AgRP, while in the POMC/CART neurons; it enhances the expression of POMC and CART. Therefore, in both sets of neurons, leptin acts to reduce food intake, making the body leaner (Chen *et al*., 2006). Leptin receptor may act as a negative regulator of Leptin activity and it may maintain a pool of available bioactive Leptin by binding and delaying its clearance from circulation (Chen *et al*., 1996).

The postulated leptin resistance is one major target in the search for a better understanding of obesity and the development of pharmacological tools to treat this spreading disease (Chen *et al*., 1996). Leptin resistance could arise from certain defects in the leptin signaling cascade. The problem in these individuals may be related to a lack of binding of the leptin protein to its receptor. Insight of three dimensional (3D) structure of a protein are of great assistance when planning experiments aimed at the understanding of protein function and during the drug design process. Knowledge of the three-dimensional structure of LEPR would greatly advance the development of drugs targeting of this molecule. The aim of the present study is to derive a putative three-dimensional structure for LEPR based on the crystal structure of GP130 of *H. sapiens* (PDB code: 1BQU) its validation and active site analysis.

MATERIALS AND METHODS

Protein sequence of *H. sapiens* Leptin receptor (LEPR) was retrieved through NCBI (http://www.ncbi.nlm.nih.gov/) using Entrez search tool and taken as target sequence. The record was downloaded and the protein sequence was stored in FASTA format, in text file. The modeling of 3D structure of LEPR followed a stepwise procedure, starting with a template structure search. Template selection was done using BLASTP (Altschul *et al*., 1997) for the query sequence against PDB (Protein Data Bank) available at NCBI**.** Homology percent was calculated with the help of GeneBee tool to select the best homolog among the selected templates. Model building was performed using the program MODELLER9v4 with multiple cycles of refinement with conjugate gradient minimization and molecular dynamics with simulated annealing. Several models at various refinements level and library schedules were generated. The validation for predicted structure models was performed by using PROCHECK (Laskowski *et al*., 1996) and energy minimization performed by Verify3D (Bowie *et al*., 1991). The overall stereochemical quality of the protein was assessed by Ramchandran plot analysis (Ramachandran *et al*., 1963). The model structure was visualized and superimposed with template using Swiss PDBviewer. Active site analysis was carried out using WHAT IF (http://swift.cmbi.ru.nl/servers) server.

RESULTS AND DISCUSSION

BLAST search was performed for *H. sapiens* LEPR across protein databases for the proteins with similar sequence and known 3D structure and significant similarities were found with several proteins belonging to the Fibronectin type III family. All proteins structures having identity more than 25% with LEPR in BLASTP search against Protein Data Bank (PDB) was stored along with their related scores, E values, percentage identity calculated excluding low-complexity regions of the sequences (Table 1). Among them five proteins 1bp3B, 1bquB, 1cd9B, 1uc6A, 1i1rA were selected for the further procedure. Homology percent was calculated with the help of GeneBee tool to select the best homolog among the selected templates (Table 2).

Table 1: Proteins found with BLAST search producing significant alignments with LEPR against PDB

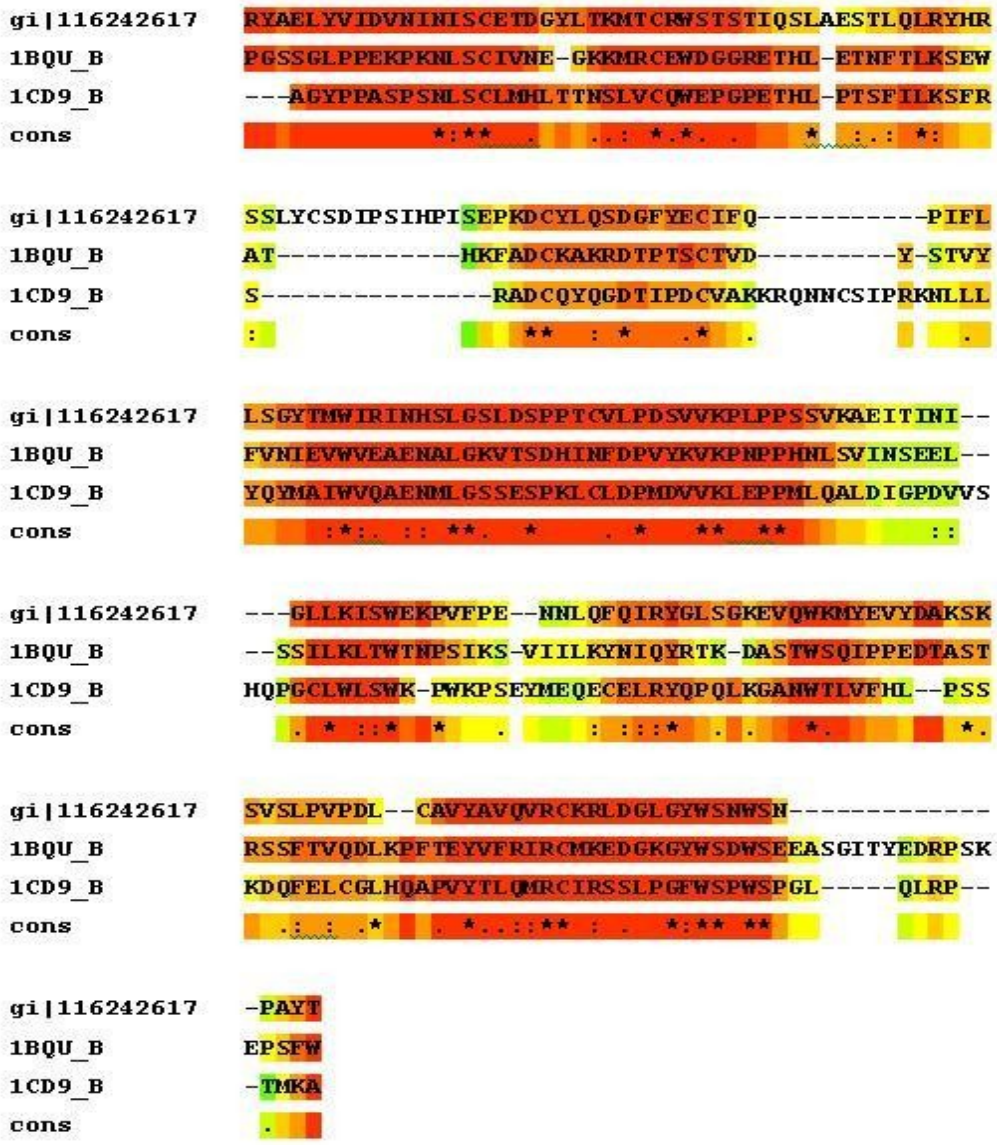
DB ID	Source	Identity (%)	E-value
2ERJ_B	mol:protein length:219 Interleukin 2 receptor beta chain	40	0.61
2B5I_B	mol:protein length:214 Interleukin 2 receptor beta chain	40	0.61
2ERJ_F	mol:protein length:219 Interleukin-2 receptor beta chain	40	0.61
1GCF_A	mol:protein length:109 GRANULOCYTE COLONY STIMULATING FACTOR RECEPTO	29	0.61
1CTO_A	mol:protein length:109 GRANULOCYTE COLONY STIMULATING FACTOR RECEPTOR	29	0.61
2YRZ_A	mol:protein length:118 Integrin beta 4	29	0.073
1BJ8_A	mol:protein length:109 GP130	28	2e-05
1EGJ_A	mol:protein length:101 CYTOKINE RECEPTOR COMMON BETA CHAIN PRECURSOR	28	0.47
1BJ8_A	mol:protein length:109 GP130	28	2e-05
1BP3_B	mol:protein length:211 PROTEIN (PROLACTIN RECEPTOR)	27	5e-04
3D48_R	mol:protein length:211 Prolactin receptor	27	5e-04
1UC6_A	mol:protein length:109 Ciliary Neurotrophic Factor Receptor alpha	26	0.007
1PGR_B	mol:protein length:215 PROTEIN (G CSF RECEPTOR)	25	2e-08
1PGR_D	mol:protein length:215 PROTEIN (G CSF RECEPTOR)	25	2e-08
1PGR_F	mol:protein length:215 PROTEIN (G CSF RECEPTOR)	25	2e-08
1PGR_H	mol:protein length:215 PROTEIN (G CSF RECEPTOR)	25	2e-08
1BQU_A	mol:protein length:215 PROTEIN (GP130)	25	3e-09
1BQU_B	mol:protein length:215 PROTEIN (GP130)	25	3e-09
1CD9_B	mol:protein length:215 PROTEIN (G-CSF RECEPTOR)	25	2e-08
1CD9_D	mol:protein length:215 PROTEIN (G-CSF RECEPTOR)	25	2e-08
1PVH_C	mol:protein length:201 Interleukin 6 receptor beta chain	25	3e-07
1PVH_A	mol:protein length:201 Interleukin 6 receptor beta chain	25	3e-07
2ED9_A	mol:protein length:124 Netrin receptor DCC	25	0.12
1C8P_A	mol:protein length:102 CYTOKINE RECEPTOR COMMON BETA CHAIN	25	0.16
2DJS_A	mol:protein length:108 Ephrin type B receptor 1	25	3.1

Table 2: Selected templates from MODELLER along with energies

Software	PDB Code	E- value
MODELLER	1bp3B	0.26E -04
	1bquB	0.26E-09
	1cd9B	0.56E-09
	1uc6A	0.39E-03
	1i1rA	0.84E-06

Among the selected templates Interleukin-2 receptor alpha chain from *H. sapiens* (PDB code: 2erj) has 40% identity in BLAST search but high E value and less homologues. On the other hand GP130 (PDB code: 1bqub) and Granulocyte Colony-Stimulating Factor (PDB code: 1cd9b) show more homology with low E Value even sequence identity is low. Multiple sequence of 1BQUB, ICD9B along with leptin sequence was performed (Figure 1). On the basis of these results, we can conclude that LEPR protein may be folded similarly to these proteins. Therefore, in the present study we used GP130 (PDB code: 1bqub) as templates to predict the 3D structure of LEPR by using the comparative modeling strategy.

Figure 1: Multiple sequence alignment between target and template Sequences



MODELLER 9V4 was used for generation of models and a total of ten models were generated (Table 3). The model with lowest objective function was selected as the best and subjected to "internal" evaluation of self-consistency checks such as stereochemical check to find the deviations from normal bond lengths, dihedrals and non-bonded atom-atom distances. The comparable Ramachandran plot characteristic and the goodness factors confirm the quality of the modeled structure The PROCHECK analysis on the stereochemical quality of the 10 models resulting from the modeling procedures were performed. On the basic of results MODEL 6 was selected as the best model created in each session. The predicted model was visualized and target and template structure was imposed using Swisspdbviewer (Figure 2A, 2B). The Goodness factors (G-factors), from the PROCHECK results summary show the quality of covalent and overall bond/angle distances. The observed G-factors for the present model were -0.22 for dihedrals, -0.32 for covalent and overall -0.25. The MODEL 6 contains 88.7% of the residues in the most favored region, 11.3 % in the additional allowed and no single residue in generously allowed regions and disallowed region (Figure 3).

Table 3: Parameters for structural verification of the predicted models

Model No.	Core region in Ramachandran plot	Disallowed region in Ramachandran plot	Energy	Modeller Objective Function
Model 1	84.3%	0.5%	-20203.49609	1255.1410
Model 2	82.7%	0.5%	-20658.91406	1305.3533
Model 3	81.1%	1.1%	-20501.09961	1356.6470
Model 4	81.6%	1.1%	-20083.04492	1320.3551
Model 5	77.8%	2.7%	-19487.11523	1627.4490
Model 6	90.8%	0.5%	-16664.66211	1231.3523
Model 7	91.4%	0.5%	-16392.58984	1284.2024
Model 8	91.9%	1.1%	-16442.51172	1323.4573
Model 9	93.0%	0.5%	-16400.54297	1336.0059
Model 10	93.0%	1.1%	-16760.01172	1325.3959

Figure 2: (A) - 3D structure of predicted Leptin receptor (sequence region 421-631), (B) - Structural superimposition of target with template proteins

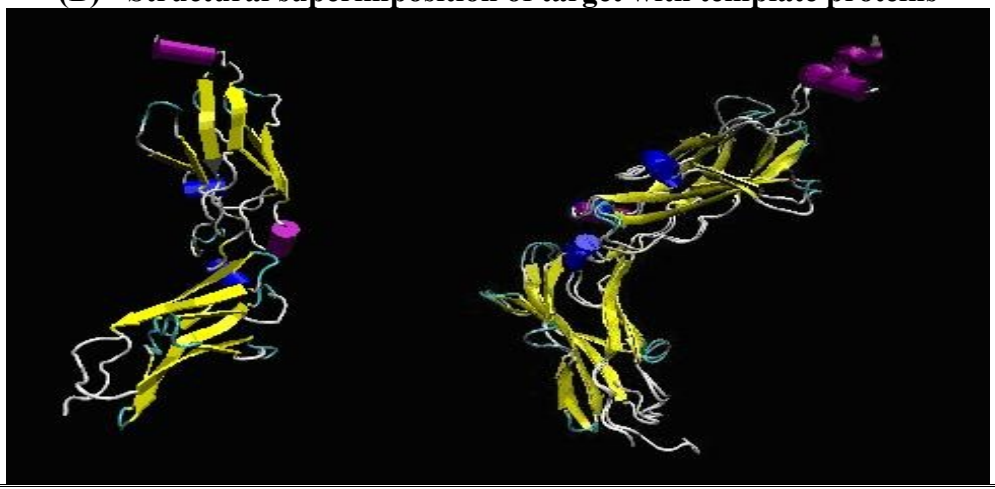
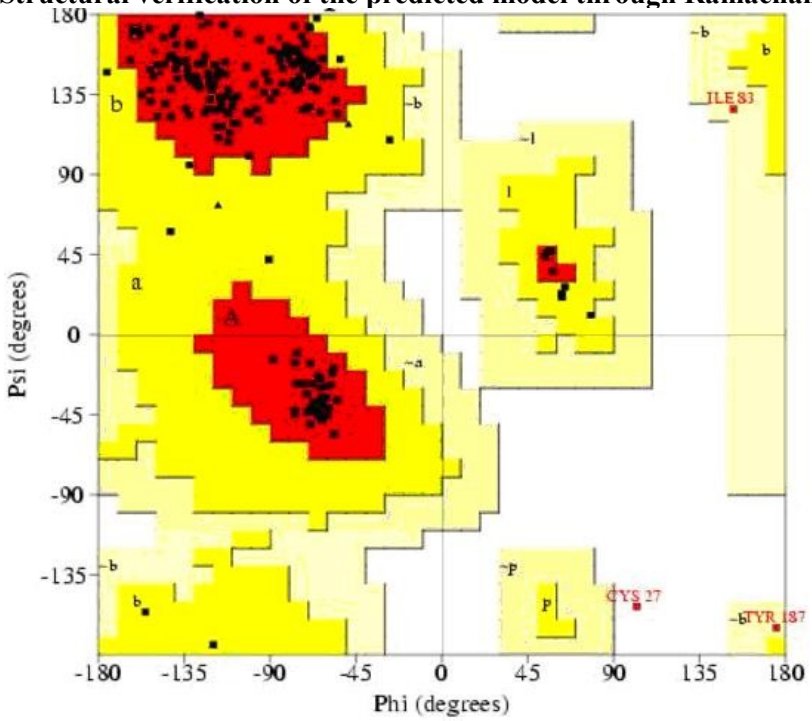


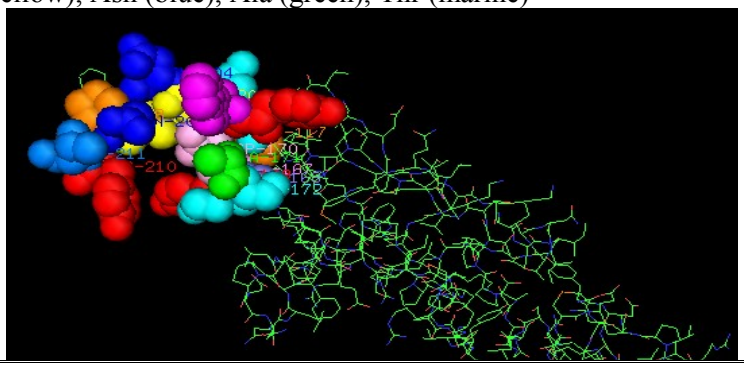
Figure 3: Structural verification of the predicted model through Ramachandran Plot



The predicted model was further analyzed to locate the residues in the active sites those provide interactions with the ligand (Figure 4). Pymol (http://www.pymol.org) and VMD (http://www.ks.uiuc.edu/Research/vmd/) were used for the analysis and for illustrating 3-dimensional structures. In whole protein sequence the active site residues are present as:
Lys536, Pro537, GLU587, Val588, Tyr589, Asp590, Ala591, Lys592, Tyr621, Trp622, Ser623, Asn624, Ser626, Asn627, Pro628, Ala629, Tyr630, Thr631.

Figure 4: Active site residues showing of predicted model 6:

Lys (cyan), Pro (orange), Glu (violet),Val (slate), Tyr (red), Asp (pink), Ala (Salmon), Trp (magenta), Ser (yellow), Asn (blue), Ala (green), Thr (marine)



CONCLUSION

Physicians need to find ways to manage obesity with the use of more effective medications. Leptin receptor has been chosen in order to provide a new target for the development of obesity drug discovery. A molecular model of the leptin receptor from *H.sapiens* is documented in this study. Using protein modeling tools, building a homology model for this protein has been accomplished and visualized. The model is believed to provide valuable insights towards the design of an inhibitor of leptin resistance for the treatment of obesity, using the provided active site location and conformations.

REFERENCES

1. Allison D.B., Fontaine K.R., Manson J.E., Stevens J., VanItallie T.B. (1999). Annual deaths attributable to obesity in the United States. JAMA, 282(16): 1530–8.
2. Altschul S.F., Madden T.L., Schaffer A.A., Zhang J., Zhang Z., Miller W., Lipman D.J., (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res., 25: 3389-3402.
3. Banks W.A., Coon A.B., Robinson S.M., Moinuddin A., Shultz J.M., Nakaoke R., Morley J.E., (2004). Triglycerides induce leptin resistance at the blood-brain barrier. Diabetes, 53(5):1253-60.
4. Barness L.A., Opitz J. M., Gilbert-Barness E., (2007). Obesity: genetic, molecular, and environmental aspects. Am. J. Med. Genet. 143A(24): 3016–34.
5. Bowie J.U., Lüthy R., Eisenberg D., (1991). A method to identify protein sequences that fold into a known three-dimensional structure.Science, 253(5016): 164-170
6. Calle E.E., Thun M.J., Petrelli J.M., Rodriguez C., Heath C.W., (1999). Body-mass index and mortality in a prospective cohort of U.S. adults. N. Engl. J. Med., 341(15): 1097–105.
7. Chen H., Charlat O., Tartaglia L.A., Woolf E.A., Weng X., Ellis S.J., et al., (1996). Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. Cell, 84: 491-5.
8. Chen K., Li F., Li J., Cai H., Strom S., Bisello A., et al., (2006). Induction of leptin resistance through direct interaction of C-reactive protein with leptin. Nat Med., 12: 425-32.
9. GreGreen E.D., Maffei M., Braden V.V., Proenca R., DeSilva U., Zhang Y., Chua S.C. Jr., Leibel R.L., Weissenbach J., Friedman J.M., (1995). The human obese (OB) gene: RNA expression pattern and mapping on the physical, cytogenetic, and genetic maps of chromosome 7. Genome Res., 5(1): 5–12.
10. Laskowski R.A., Rullmannn J.A., MacArthur M.W., Kaptein R., Thornton J.M., (1996). AQUA and PROCHECK-NMR: programs for checking the quality of protein structures solved by NMR. J Biomol. NMR., 8: 477.
11. Ramachandran G.N., Ramakrishnan C., Sasisekharan V., (1963). Stereochemistry of polypeptide chain configurations. J. Mol. Biol., 7: 95-9

****