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Analysis of insulin-like growth factor I and its receptor of an Indian major carp *labeo rohita*: an *in silico* approach

Abstract

The Insulin-like growth factor I (IGF-I) is produced from the liver by involving growth hormone through the axis of pituitary/hepatic-GH/IGF-I system. IGF-I and its receptors (IGF-IR) are expressed in various extrahepatic tissues. The circulatory IGF-I promotes the systemic body growth by effecting cells of muscle, cartilage, bones etc. Various hormones such as: growth hormone (GH), Insulin-like growth factor-I (IGF-I) and their related receptors are available in different tissues of fishes which performs the growth promoting activities. Therefore, *in silico* analysis of IGF-I and its receptor, IGF-IR was carried out in Indian major carp, *Labeo rohita* to understand physicochemical properties as well as the 3D structure of these proteins. The IGF-I and IGF-IR protein sequences (accession number AME16981.1 and AQV11106.1, respectively) were analyzed by Expasy's prot param for the physicochemical characteristics, SOPMA for prediction of secondary structures and SWISSMODEL/Workspace for template search and Swiss-Pdb-Viewer for 3D structure of the concerned proteins. The results suggested that IGF-I is an unstable, hydrophilic and basic nature of protein; whereas IGF-IR is unstable, hydrophilic and acidic in nature. The secondary structure of IGF-I showed the presence of alpha helix 32.30%, extended strands 11.18% and 6.83% beta turns along with random coiling of 49.69% whereas in IGF-IR alpha helixes are 27.03% followed by 40.26% of random coiling, extended strands with 22.12% and beta turn with 10.60%. The validation of predicted 3D structures by RAMPAGE exhibited 96.7% residues in favoured region and 3.3% in outlier region in case of IGF-I, whereas IGF-IR represented 92.2% of residues in the favoured region, 6.8% in allowed region and 1.0% in outlier region. The Ramachandran plot analysis indicated that both the model is expected to be correct in prediction. Such predicted 3D structure of IGF-I and IGF-IR can also be utilized for molecular docking and simulation studies in future. Hence, the study also reduces the gap generated due to large amount of data with available sequences and solved structures by various laboratory techniques, such as: X-ray crystallography and NMR spectroscopy, which are tedious in implication.

Keywords: insulin-like growth factor i and its receptor, *in silico* analysis, 3D structure, *labeo rohita*, Indian major carp

Volume 6 Issue 3 - 2017

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Received: May 03, 2017 | **Published:** November 01, 2017

Introduction

Insulin-like growth factor (IGF) remains in the different classes of vertebrates including birds,[1,2] reptiles, amphibians,[3] fishes[4–6] and mammals.[7] Insulin-like growth factor I (IGF-I) is a protein comprising two major forms, IGF-I and IGF-II.[8,9] The IGF-I is produced from the liver Sjogren et al.,[10] by the influence of growth hormone involving the axis of pituitary/hepatic system-GH/IGF-I system.[11,12] Further, the IGF-I[13] and its receptors (IGF-IR) are also expressed in various extrahepatic regions.[14] The circulatory IGF-I promotes the systemic body growth by effecting cells of muscle, cartilage, bones etc.[15] The transmembrane receptor insulin-like growth factor I (IGF-IR) is involved in exerting the functional role of IGF-I for the promotion of growth by effecting the anabolic reactions of the physiological system.[16] The binding of IGF-IR to IGF-I or IGF-II, leads to activation of either RAS/RAF/MEK/ERK or PI3K/AKT signaling pathways for promoting the cell proliferation and anti-apoptotic activities.[17] A crosstalk of insulin/IGF-I and GPCR signaling mechanism can create suppression of apoptosis in certain physiological conditions.[18]

Growth rate increase by manipulation of genes,[19,20] as well as through selective breeding technologies Lind CE et al.,[21] has potential effects in the economic trait due to the high fish food demand. Therefore, molecular studies on various growth promoting factors in the physiological system of several fish species have been undertaken since a decade. Various genes of fishes, such as: growth hormone (GH), Insulin-like growth factor-I (IGF-I) and the related hormone receptors are engaged for the promotion of growth. Molecular studies on the dominant hormone responsible for body growth i.e. the growth hormone is subject of extensive research for last few years.[22–24]

The involvement of IGF-II during the early embryogenesis of fishes[6,25–27] followed by declination phase of the level and subsequent increase of IGF-I level in the adult phases showed growth promoting effects in the life cycle of fishes.[28,29] The availability of IGF-I receptors have been detected in different tissues of fishes.[6] The potential involvement of IGF-I and IGF-IR in fish growth creates a need to understand the 3D structures of the query proteins for realizing their interactive activity during the fish growth and development. Therefore, an attempt was undertaken in the present study for conducting the *in silico* analysis of Insulin-like growth factor-I (IGF-I) and its receptor, IGF-IR in Indian major carp, *Labeo rohita* (Hamilton, 1822). The study will highlight the various physicochemical parameters assessment and 3D structure information of IGF-I and IGF-IR proteins, which will

help further to understand the molecular basis of their role in *Labeo rohita*. Further, 3D structure prediction by X-ray crystallography and NMR spectroscopy is a time-consuming and tedious methodology, whereas *in silico* method of prediction reduces this effort.

Materials and methods

The amino acid sequence of insulin-like growth factor I (IGF-I) and insulin-like growth factor I receptor (IGF-IR) of *Labeo rohita* were retrieved from National Center for Biotechnology Information (NCBI) heaving the accession number AME16981.1 and AQV11106.1 respectively. The FASTA format of the sequences were downloaded and used for further analysis. Physicochemical properties like: molecular weight, theoretical pI, % total number of negative and positive residue, the composition of amino acids, instability index, grand average of hydropathicity (GRAVY) of IGF-I and IGF-IR protein were calculated using Expasy's program server. Self-Optimized Prediction Methods with Alignment (SOPMA) were employed to understand the secondary structure of the proteins. Template selection searches were performed by using SWISSMODEL/Workspace. For homology modeling, template 1tgr.1.A and 3lvp.1.A along with sequence identity of 86.54% and 89.87% were selected respectively for IGF-I and IGF-IR. Finally, the structures were predicted by using Swiss-Pdb-Viewer. The predicted structures were then validated in Ramachandran plot by using RAMPAGE server.

Results and discussion

The neuroendocrine regulation of growth and related factors potentially involves insulin-like growth factor (IGF) and its receptors.[27] Extensive studies on the IGF signaling mechanism has been studied in teleosts.[30,31] Studies on zebra fish showed requirements of IGF signaling for development during vertebrate embryonic condition as well as in germ cell migration and survival.[32,33] Insulin-like growth factors (IGFs) completes the growth promotion by acting in a paracrine or autocrine fashion on skeletal muscle.[34] Various studies on lower vertebrate organisms including fishes suggested the role the IGF system in development neuroendocrine regulation of growth and also insights its perspectives in the evolution.[27] So to understand the role of IGF system in piscine endocrinology, an initiative was taken here to analyze the Insulin-like growth factor I (IGF-I) and its receptor (IGF-IR) following the *in silico* methodologies for an Indian major carp species *Labeo rohita*.

Prediction of physicochemical properties:

The physicochemical properties of IGF-I and IGF-IR protein were analyzed by Expasy's protParam server. IGF-I is a 161 amino acid containing polypeptide showing estimated molecular weight 17872.41kDa and theoretical isoelectric point (pI) is 9.10 which presents basic nature of the protein. The amino acid composition of IGF-I sequence showed the maximum presence of Serine (9.3%) and minimum presence of Tryptophan and Isoleucine (0.6%).The total number of positive and negatively charged residues of IGF-I are (Asp+Glu)-13 and (Arg+Lys)-22, respectively. The estimated instability index (ll) of the IGF-I protein is 48.82 which classifies the protein as unstable. Aliphatic index (53.85) of the IGF-I protein measures its thermostability along with the relative volume occupied by aliphatic side chains. The negative value (-0.498) of the grand average of hydropathicity (GRAVY) indicates that IGF-I is a hydrophilic protein.

On the other hand, the receptor protein accepting the IGF-I contains 1406 amino acid with estimated molecular weight 157996.14kDa and theoretical isoelectric point (pI) 5.94 which represents acidic nature of the protein. Leucine (8.4%) is the maximum number and tryptophan (1.5%) is the minimum of amino acid present in the polypeptides of IGF-IR. Comparative analyses of amino acid compositions of both IGF-I and IGF-IR were presented in the Table 1. In IGF-IR, (Asp+Glu)-173 and (Arg+Lys)-154 respectively represents the total number of positive and negatively charged residues. The IGF-IR instability index (ll) is 49.10 which demarcates it more unstable protein compared to IGF-I. Aliphatic index and GRAVY for IGF-IR measures 76.07 and -0.373 respectively (Table 2).

Table 1 Comparative analyses of amino acid compositions of both IGF-I and IGF-IR

Amino acid composition	IGF-I		IGF-IR	
	Number of amino acids	% of amino acids	Number of amino acids	% of amino acids
Ala (A)	7	4.3	82	5.8
Arg (R)	14	8.7	74	5.3
Asn (N)	4	2.5	70	5
Asp (D)	6	3.7	73	5.2
Cys (C)	12	7.5	45	3.2
Gln (Q)	4	2.5	47	3.3
Glu (E)	7	4.3	100	7.1
Gly (G)	14	8.7	93	6.6
His (H)	8	5	29	2.1
Ile (I)	1	0.6	72	5.1
Leu (L)	12	7.5	118	8.4
Lys (K)	8	5	80	5.7
Met (M)	4	2.5	43	3.1
Phe (F)	7	4.3	56	4
Pro (P)	10	6.2	89	6.3

Table Continued....

Amino acid composition	IGF-I		IGF-IR	
	Number of amino acids	% of amino acids	Number of amino acids	% of amino acids
Ser (S)	15	9.3	109	7.8
Thr (T)	13	8.1	70	5
Trp (W)	1	0.6	21	1.5
Tyr (Y)	4	2.5	50	3.6
Val (V)	10	6.2	85	6

Table 2 Ramachandran plot of IGF-I and IGF-IR showing % of allowed, favorable and outlier regions

Name of the proteins	Favoured region	Allowed region	Outlier region
IGF-I	96.7%	--	3.3%
IGF-IR	92.2%	6.8%	1.0%

Secondary structure prediction

The secondary structure of IGF-I and IGF-IR were predicted by using SOPMA. The predictions of secondary structures in SOPMA were conducted along with the default parameters of window width 17, similarity threshold 8 and number of states 4 (Combet et al., 2000). IGF-I secondary structure prediction suggested presence of alpha helix (32.30%) with 52 and extended strands (11.18%) with 18 numbers. Random coils are predominantly higher (49.69%) along with 80 in IGF-I, whereas comparatively lesser (6.83%) beta turns are observed in the predicted structure composed of 11 numbers (Figure 1).

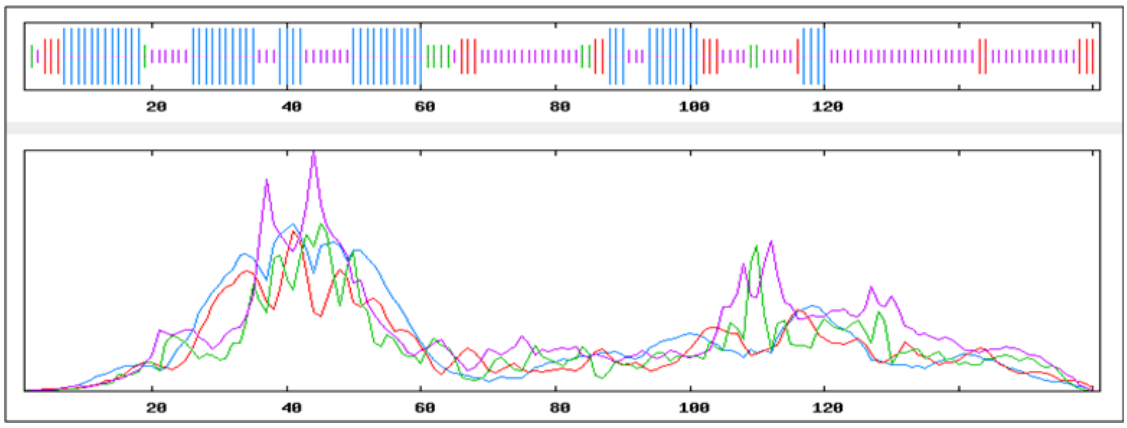

Figure 1 Graphical representation of secondary structure of IGF-I.

The secondary structure analysis of IGF-IR showed alpha helixes are higher in percentage rate (27.03%) with 380 followed by extended strands with 311 (22.12%) numbers. Predominantly, maximum percentage (40.26%) of secondary structure lies as a random coiling with 566, whereas 149 (10.60%) numbers are utilized for the preparation of beta turn in the protein (Figure 2).

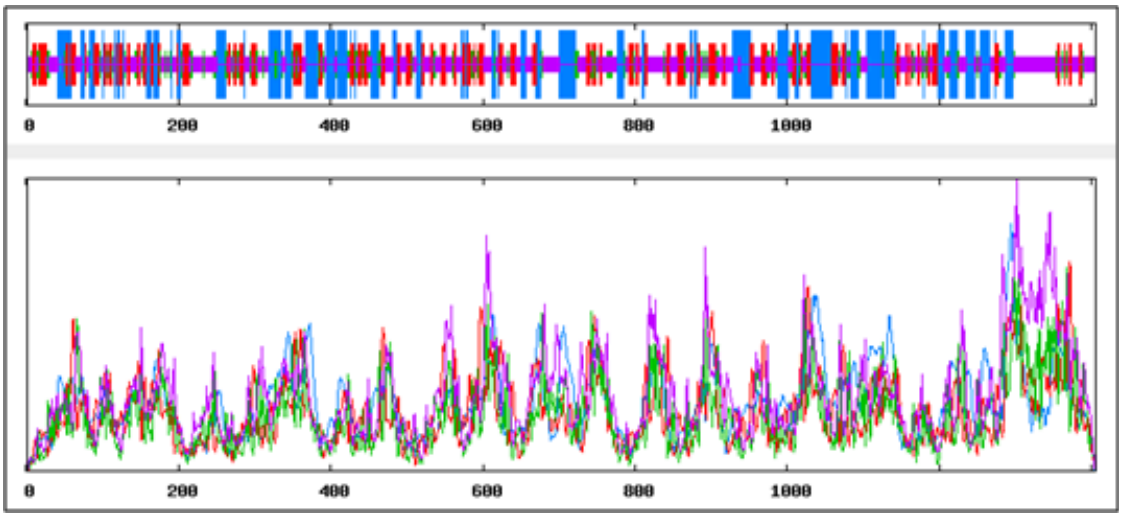

Figure 2 Graphical representation of secondary structure of IGF-IR.

Template selection, 3D structure prediction by Homology modeling and validation of the model

For homology modeling, template 1tgr.1.A and 3lvp.1.A along with sequence identity of 86.54% and 89.87% were selected respectively for IGF-I and IGF-IR by using SWISSMODEL/ Workspacec. The experimental structure used for the prediction of IGF-I and IGF-IR model as template were the monomers of X ray diffraction method along with 1.4Å and 3.0Å (Figure 3) (Figure 4). By using Swiss-PdbViewer, the 3D structure of IGF-I and IGF-IR were predicted on the basis of homology modeling.[35] The structural alignments were generated from Swiss model server by aligning sequences of IGF-I and IGF-IR with respective template proteins (Figure 5 & 6). Model quality was estimated by assessing the QMEAN score, which stands for qualitative model energy analysis is composite scoring function describing the major geometrical aspects of protein structures. QMEAN was tested on several standard decoy sets including a molecular dynamics simulation decoy set as well as on a comprehensive data set. QMEAN shows a statistically significant improvement over nearly all quality measures describing the ability of the scoring function to identify the native structure and discriminate good from bad models.[36] The conformations of the predicted 3D structures (Figure 7 & 8) of the proteins were validated by Ramachandran plot (phi/psi). The stereochemical analysis[37] of IGF-I by RAMPAGE showed number of residues in favoured region is 96.7% and in outlier region is 3.3% (Figure 9), whereas in case of IGF-IR, the RAMPAGE analysis of suggested 92.2% of residues remains in the favoured region, 6.8% in allowed region and 1.0% in outlier region (Figure 10), which indicates that both of the models are likely to be correct in prediction.[38]

Figure 3 Result of template search for IGF-I.

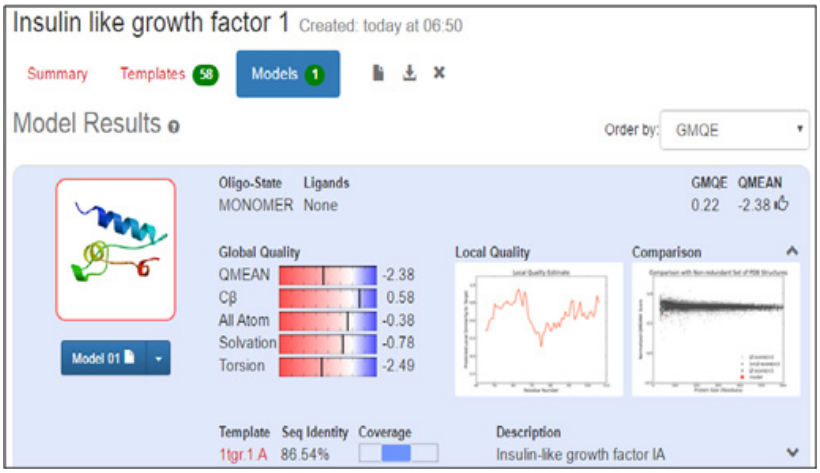

Figure 4 Result of template search for IGF-IR.

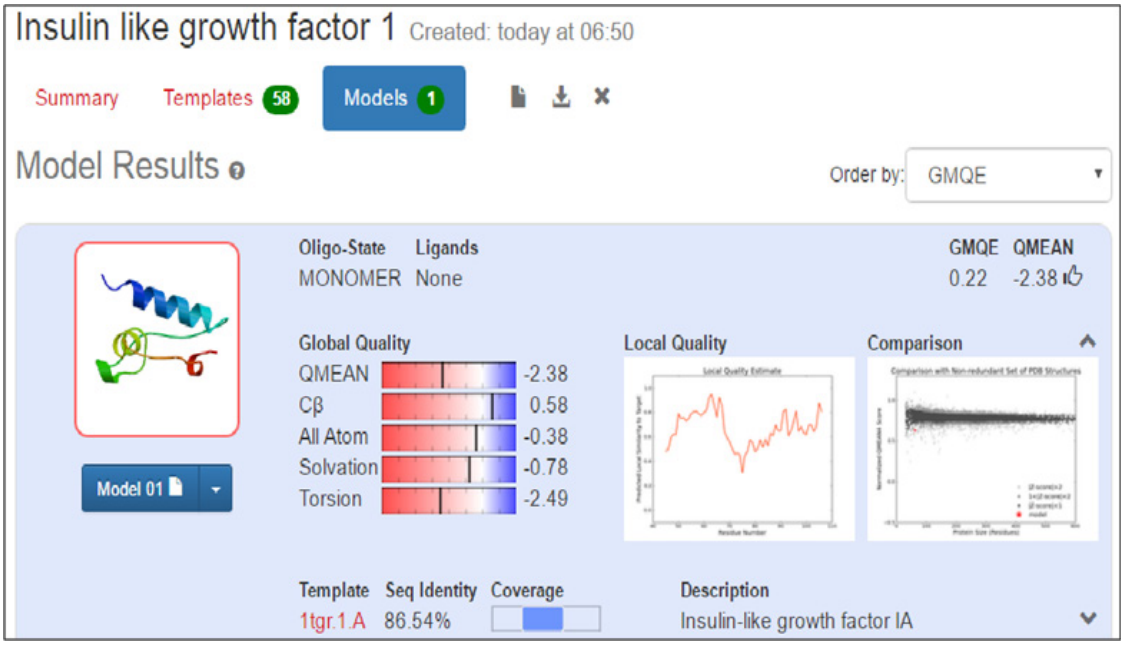

Figure 5 Modeling result of IGF-I.

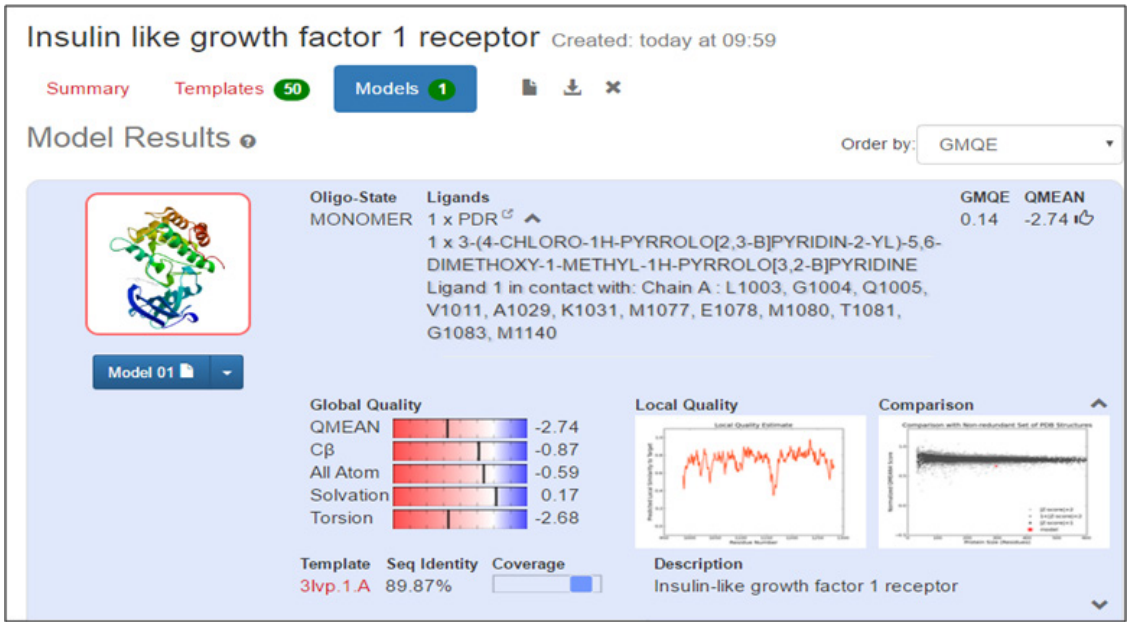

Figure 6 Modeling result of IGF-IR.

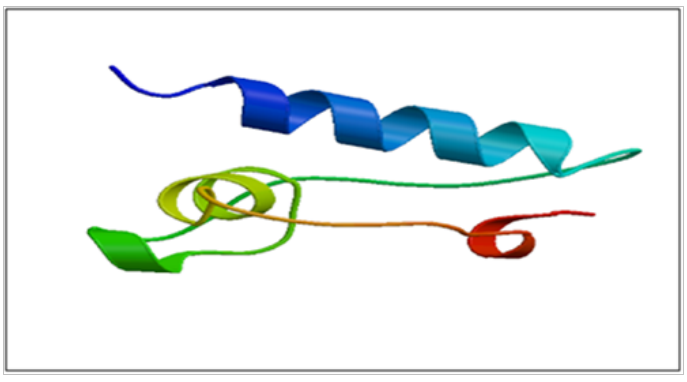

Figure 7 Predicted 3D structure of IGF-I.

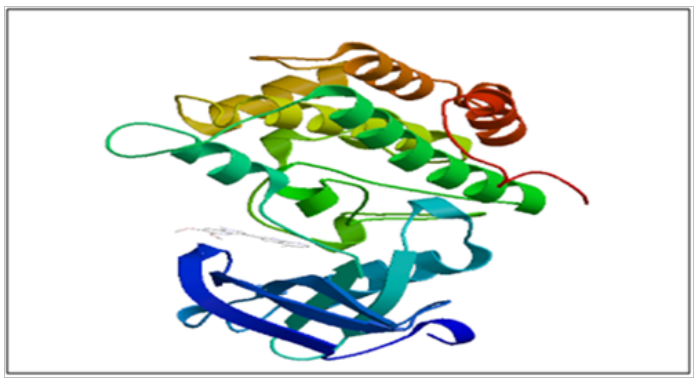

Figure 8 Predicted 3D structure of IGF-IR.

Citation: Acharjee S, Upadhyay AD, Roy AK, et al. Analysis of insulin-like growth factor I and its receptor of an Indian major carp *labeo rohita*: an *in silico* approach. *MOJ Proteomics Bioinform.* 2017;6(3):287–293. DOI: 10.15406/mojpb.2017.06.00195

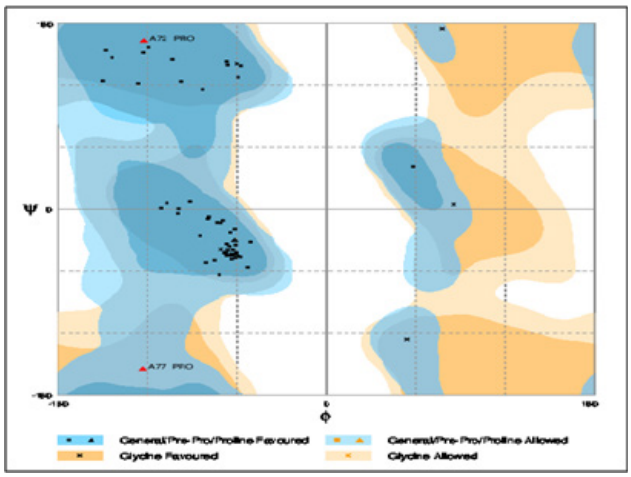

Figure 9 Ramchandran plot for the predicted 3D structure of IGF-I.

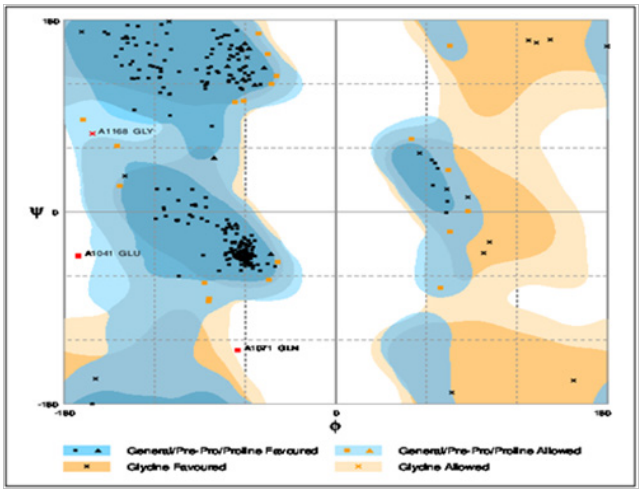

Figure 10 Ramchandran plot for the predicted 3D structure of IGF-IR.

Conclusion

The study described various structural and physicochemical parameters of IGF-I and IGF-IR protein; whereas the predicted 3D structure highlighted a conceptual direction about the receptor protein and its ligand protein in *Labeo rohita,* which might help to understand the interaction of the concerned proteins involved in body growth. Further, X-ray crystallography and NMR spectroscopy are most convenient method of 3D structure prediction, but requires enormous time and financial support, however also a tedious method in implication. Moreover, the application of such bioinformatics based tools minimizes the gap generated due to large amount of data with available sequences and solved structure. Based on present findings, it could be concluded that the IGF-I is an unstable, hydrophilic and basic nature of protein; whereas IGF-IR is unstable, hydrophilic and acidic in nature. The secondary structure of the analyzed proteins suggested presence of alpha helix 32.30%, extended strands 11.18% and 6.83% beta turns along with random coiling of 49.69% in case of IGF-I. But the receptor of IGF-I (IGF-IR) showed alpha helixes 27.03% followed by 40.26% of random coiling, extended strands with 22.12% and beta turn with 10.60%. Moreover, it might be resolved that the 3D structure of IGF-I exhibited 96.7% residues in favoured region and 3.3% in outlier region, whereas IGF-IR represented 92.2% of residues in the favoured region, 6.8% in allowed region and 1.0% in outlier region, indicating the model is expected to be correct in prediction. The predicted 3D structure of IGF-I and IGF-IR protein can also be further utilized for molecular docking and simulation studies in near future. Further studies on Comparative modeling along with docking and simulation may be carried out in future.

Acknowledgements

Authors are thankful to the Dean, College of Fisheries, Central Agricultural University (I), Lembucherra, Tripura (W) for encouragement and moral support. The financial assistance through the project "Establishment of Bioinformatics Infrastructure Facility for Biology Teaching through Bioinformatics" by the Department of Biotechnology, New Delhi, and Govt. of India is duly acknowledged.

Conflict of interest

The author declares no conflict of interest.

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