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

\textbf{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, \textit{in silico} analysis of IGF-I and its receptor, IGF-IR was carried out in Indian major carp, \textit{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 Exapy's prot param for the physicochemical characteristics, SOPMA for prediction of secondary structures and SWISSMODEL_workspace for template search and Swiss-Pdb-View 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 helices 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.

\textbf{Keywords:} insulin-like growth factor i and its receptor, \textit{in silico} analysis, 3D structure, \textit{labeo rohita}, Indian major carp

\textbf{Introduction}

Insulin-like growth factor (IGF) remains in the different classes of vertebrates including birds,\textsuperscript{1,2} reptiles, amphibians,\textsuperscript{3} fishes\textsuperscript{4-6} and mammals.\textsuperscript{7} Insulin-like growth factor I (IGF-I) is a protein comprising two major forms, IGF-I and IGF-II.\textsuperscript{1,4} The IGF-I is produced from the liver Sjögren et al.,\textsuperscript{8} by the influence of growth hormone involving the axis of pituitary/hepatic system-GH/IGF-I system.\textsuperscript{9,10} Further, the IGF-I\textsuperscript{14} and its receptors (IGF-IR) are also expressed in various extrahepatic regions.\textsuperscript{14} The circulatory IGF-I promotes the systemic body growth by effecting cells of muscle, cartilage, bones etc.\textsuperscript{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.\textsuperscript{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.\textsuperscript{17} A crosstalk of insulin/IGF-I and GPCR signaling mechanism can create suppression of apoptosis in certain physiological conditions.\textsuperscript{18}

Growth rate increase by manipulation of genes,\textsuperscript{19,20} as well as through selective breeding technologies Lind CE et al.,\textsuperscript{3} 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.\textsuperscript{13,21-24} The involvement of IGF-II during the early embryogenesis of fishes\textsuperscript{6,20-22} 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.\textsuperscript{26,27} The availability of IGF-I receptors have been detected in different tissues of fishes.\textsuperscript{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 \textit{in silico} analysis of Insulin-like growth factor-I (IGF-I) and its receptor, IGF-IR in Indian major carp, \textit{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 \textit{Labeo rohita}. Further, 3D structure prediction by X-ray crystallography and NMR spectroscopy is a time-consuming and tedious methodology, whereas \textit{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 \textit{Labeo rohita} were retrieved from National Center for Biotechnology Information (NCBI) having 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 pl, % total number of negative and positive residue, the composition of amino acids, instability index, grand average of hydrophobicity (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.\textsuperscript{27} Extensive studies on the IGF signaling mechanism has been studied in teleosts.\textsuperscript{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.\textsuperscript{32,33} Insulin-like growth factors (IGFs) completes the growth promotion by acting in a paracrine or autocrine fashion on skeletal muscle.\textsuperscript{34} Various studies on lower vertebrate organisms including fishes suggested the role the IGF system in development neuroendocrine regulation of growth and also insight its perspectives in the evolution.\textsuperscript{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 \textit{in silico} methodologies for an Indian major carp species \textit{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 (II) 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 (II) 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 |

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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%          |

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     |

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.

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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/ Workspace. 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. 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. The conformations of the predicted 3D structures (Figure 7 & 8) of the proteins were validated by Ramachandran plot (phi/psi). The stereochemical analysis 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.
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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.

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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 coil of 49.69% in case of IGF-I. But the receptor of IGF-I (IGF-IR) showed alpha helices 27.03% followed by 40.26% of random coil, 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.

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Conflict of interest

The author declares no conflict of interest.

References

1. Ralphs JR, Wylie L, Hill DJ. Distribution of insulin–like growth factor peptides in the developing chick embryo. Development. 1990;109(1):51–58.
2. Radecki SV, Capdevielle MC, Buonomo FC, et al. Ontogeny of insulin–like growth factors (IGF–I and IGF–II) and IGF binding proteins in the chicken following hatching. Gen Comp Endocrinol. 1997;107(1):109–117.
3. Reinecke M, Broger I, Brun R, et al. Immunohistochemical localization of insulin–like growth factor I and II in the endocrine pancreas of birds, reptiles, and amphibia. Gen Comp Endocrinol. 1995;100(3):385–396.
4. Cao QP, Duguay SJ, Plisetskaya E, et al. Nucleotide sequence and growth hormone regulated expression of salmon insulin–like growth factor I mRNA. Mol Endocrinol. 1989;3(12):2005–2010.
5. Shambrell MJ, Chen TT. Identification of a second insulin–like growth factor in a fish species. Proc Natl Acad Sci USA. 1992;89(19):8913–8917.
6. Greene MW, Chen TT. Quantitation of IGF–I, IGF–II, and multiple insulin receptor family member messenger RNAs during embryonic development in rainbow trout. Mol Reprod Dev. 1999;54(4):348–361.
7. Rincon M, Rudin E, Barzilai N. The insulin/IGF–1 signaling in mammals and its relevance to human longevity. Exp Gerontol. 2005;40(11):873–877.
8. Hummel RE. Insulin–like growth factors I and II. Eur J Biochem. 1990;190(3):445–462.
9. Sara VR, Hall K. Insulin–like growth factors and their binding proteins. Physiol Rev. 1990;70(3):591–614.
10. Sjogren K, Liu JL, Blad K, et al. Liver-derived insulin–like growth factor I (IGF–I) is the principal source of IGF–I in blood but is not required for postnatal body growth in mice. Proc Natl Acad Sci USA. 1999;96(12):7088–7092.
11. Bjornsson BT, Johansson, Benedet VS, t al. Growth hormone endocrinology of salmonids: regulatory mechanisms and mode of action. Fish Physiol Biochem. 2004;27(3–4):227–242.
12. Butler AA, Le Roith D. Control of growth by the somatotrophic axis: growth hormone and the insulin–like growth factors have related and independent roles. Ann Rev Physiol. 2001;63:141–164.
13. Reinecke M, Schmid A, Ermatinger R, et al. Insulin–like growth factor I in the teleost Oreochromis mossambicus, the tilapia: gene sequence, tissue expression, and cellular localization. Endocrinology. 1997;138(9):3613–3619.
14. Radaelli G, Domenechini C, Arrighi S, et al. Localization of IGF–I, IGF–I receptor, and IGFBP–2 in developing Umbrina cirrosa (Pisces:Osteichthyes). Gen Comp Endocrinol. 2003;130(3):232–244.

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15. Yakar S, Rosen CJ, Beamer WG, et al. Circulating levels of IGF–1 directly regulate bone growth and density. J Clin Invest. 2002;110(6):771–781.

16. Stewart EH, Rotwein P. Growth, differentiation, and survival: multiple physiological functions for insulin–like growth factors. Physiol Rev. 1996;76(4):1005–1026.

17. Rongshi Li, Pourpak A, Morris SW. Inhibition of the Insulin–like Growth Factor–1 Receptor (IGF1R) tyrosine kinase as a novel cancer therapy approach. J Med Chem. 2009;52(16):4981–5004.

18. Rozengurt E, Sinnett-Smith J, Kisfalvi K. Crosstalk between insulin/insulin–like growth factor–1 receptors and G protein–coupled receptor signaling systems: a novel target for the antidiabetic drug metformin in pancreatic cancer. Clin Cancer Res. 2010;16(9):2505–2511.

19. Zuo YZ, Yong HS. Embryonic and genetic manipulation in fish. Cell Res. 2000;10(1):17–27.

20. Erundu ES, Akinrotimi OA, Gabriel UU. Genetic manipulation for enhanced aquaculture production in Nigeria. Bioscience Research Journal. 2011;23:2.

21. Lind CE, Ponzoni RW, Nguyen NH, et al. Selective Breeding in Fish and Conservation of Genetic Resources for Aquaculture. Reprod Dom Anim. 2012;47(Suppl 4):255–263.

22. Yada T. Growth hormone and fish immune system. Gen Comp Endocrinol. 2007;152(2–3):353–358.

23. Canosa LF, Chang JP, Peter RE. Neuroendocrine control of growth hormone in fish. Gen Comp Endocrinol. 2007;151(1):1–26.

24. Reinecke M, Bjornsson BT, Dickho WW, et al. Growth hormone and insulin–like growth factors in fish: Where we are and where to go. Gen Comp Endocrinol. 2005;142(1–2):20–24.

25. Ndez EM, Planas JV, Castillo J, et al. Identification of a Type II Insulin–Like Growth Factor Receptor in Fish Embryos. Endocrinology. 2001;142(3):1090–1097.

26. Greene MW, Chen TT. Temporal expression pattern of insulin–like growth factor mRNA during embryonic development in a teleost, rainbow trout (Onchorhyncus mykiss). Mol Mar Biol Biotech. 1997;6(2):144–151.

27. Wood AW, Duan C, Berms HA. Insulin–Like Growth Factor Signaling in Fish. Int Rev Cytol. 2005;243:215–285.

28. Parrot V, Moiseeva EB, Gozes Y, et al. Ontogeny of the insulin–like growth factor system (IGF–I, IGF–II, and IGF–IR) in gilthead seabream (Sparus aurata): expression and cellular localization. Gen Comp Endocrinol. 1999;116(3):445–460.

29. Duguay SJ, Lai–Zhang J, Steiner DF, et al. Developmental and tissue–regulated expression of IGF–I and IGF–II mRNAs in Sparus aurata. J Mol Endocrinol. 1996;16(2):123–132.

30. Mancera JM, McCormick SD. Osmoregulatory actions of the GH/IGF axis in non–salmonid teleosts. Comparative Biochemistry and Physiology. 1998;121(1):43–48.

31. Wang DS, Jiao B, Hu C, et al. Discovery of a gonad–specific IGF subtype in teleost. Biochem Biophys Res Commun. 2008;367(2):336–341.

32. Schlueter PJ, Peng G, Westerfield M, et al. Insulin–like growth factor signaling regulates zebrafish embryonic growth and development by promoting cell survival and cell cycle progression. Cell Death Differ. 2007;14(6):1095–1105.

33. Schlueter PJ, Sang X, Duan C, et al. Insulin–like growth factor receptor 1b is required for zebra fish primordial germ cell migration and survival. Dev Biol. 2007;305(1):377–387.

34. Wang Y, Bikle DD, Chang W. Autocrine and Paracrine Actions of IGF–1 Signaling in Skeletal Development. Bone Res. 2013;1(3):249–259.

35. Guex N, Peitsch MC, Schwede T. Automated comparative protein structure modeling with SWISS–MODEL and Swiss–Pdb Viewer: a historical perspective. Electrophoresis. 2009;1:S162–S173.

36. Benkart P, Biasini M, Schwede T. Toward the estimation of the absolute quality of individual protein structure models. Bioinformatics. 2011;27(3):343–350.

37. Filiz E, Koc I. In silico sequence analysis and homology modeling of predicted beta–amylase 7–like protein in Brachypodium distachyon L. J BioSci Biotech. 2014;3(1):61–67.

38. Combet C, Blanchet C, Geourjon C, et al. NPS@network protein sequence analysis. Trends Biochem Sci. 2000;25(3):147–150.

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