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Comparative Studies on Kisspeptin Receptor and their Physicochemical Characterization

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A B S T R A C T

The kisspeptins are peptide hormones, having a critical role in the regulation of the hypothalamic–pituitary–gonadal axis, and important for reproduction. Kisspeptin which is the product of the *Kiss1* gene and its receptor (GPR54 or Kiss1r) has now emerged as one of the key players in the regulation of reproduction. In this present work comparative study of Human, Rat, Zebra fish Kisspeptin receptor has been done. Insilco tools were used to describe the physiochemical, functional and structural properties of this receptor protein. Physico-chemical properties like as molecular weight, amino acid composition, isoelectric point, charge residue, Grand average of hydropathicity, instability index are computed. The studies give insight into kisspeptin receptor.

Introduction

The human kisspeptin gene was first identified as a melanoma metastasis suppressor gene (Lee *et al.,* 1996) and with the help of inactivating mutations of its cognate receptor gene, KISS1R, which inhibited the pubertal development (Seminara *et al.,* 2003) researcher found that both gene and its receptor are essential components of the mammalian reproductive system (Kauffman 2010). The isolation of KISS1 and KISS1R gene orthologs in a different organism like as in amphibians and fish etc. The study shows that conserved reproductive function of this ligand and receptor across vertebrates, having an exception in birds (Kim *et al.,* 2012). The detailed study of kisspeptin and its receptor

are beyond the limit of this research. In this present work we try to compare the rat, human and zebrafish kisspeptin receptor with the help of *in silico* tools.

Materials and Methods

Human (Ohtaki *et al.,* 2001), Rat (Kotani *et al.,* 2001) and zebra fish (Accession number F1R5R4 and F1QZU4) Kisspeptin receptor Sequence were selected and its protein fasta file was retrieved using NCBI and was used for further analysis.

Physiochemical properties

Physiochemical properties were calculated using Protparam tool (https://web.expasy. org/protparam/) (Gasteiger *et al.,* 2005) which gives details about molecular weight, theoretical Pi, amino acid composition etc. The amino acid composition was determined by using the protparam tool.

Functional analysis and secondary structure analysis

Transmembrane regions were identified using TMHMM Server v. 2.0 server. SOPMA (Geourjon *et al.,* 1987) and GOR (Heymann *et al.,* 2008) were used for predicting the secondary structure of the protein. In these tools the presence of alpha helx, pi helix, beta bridge, beta turn etc. is determined in terms of percentage.

The prediction of the protein structure was done using GPCR-I-TASSER (Zhang *et al.,* 2015) which characterises various structural based templates from PDB database by various approaches that have various atomic based models. Ramchandran plot of the respective protein structure was determined by RAMPAGE (http://mordred. bioc.cam.ac.uk/ ~rapper/rampage.php) and the quality of the Ramchandran plot was characterised by WHAT IF SERVER (Vriend *et al.,* 1990). For Active binding site prediction COACH server were used.

Results and Discussion

In this presenter search four Kisspeptin receptor sequences were retrieved from NCBI and they are analysed computationally (Table 1). The primary structure analysis which means the Physico-chemical properties of this receptor was computed using Expasy's Prot Param tool and shown in Table 2. By analysing these we found that Alanine and Leucine amino acid are most abundant and the isoelectric point (pI) is the value at which the molecule carries no charges or/and positive negative charges is found to be equal. At a pH below their pI value of proteins carry a net positive charge; above their pI these carry a net negative charge. In our study this is highest for (Q969F8) and lowest value for F1QZU4 respectively all values in basic pH range. The Extinction coefficient (EC) at a 280nm wavelength ranged from 63675 to 67380. The calculated extinction coefficient values help us in various quantitative studies of protein-protein and protein-ligand interactions in solution. The instability index value of proteins was computed by EsPasy protparam which gives an approximation of the stability of the protein in vitro. A protein whose instability index is smaller than 40 is predicted as stable, a value above 40 predicts that the protein may be unstable. The instability indexes of this receptor are ranging from 34.23 to 48.25 (Table 2). By analysing the instability index we can conclude that except Q969F8 other are stable The aliphatic index (AI) range from 93.20 to 103.05 which is the relative volume of a protein occupied by aliphatic side chains (A, V, I and L) is regarded as a positive factor for the increase of thermal stability of globular proteins. The lower thermal stability values indicate a more flexible structure when we compare with other receptors.

Table.1 KISS receptor derived from NCBI database

ACCESSION NUMBER	SEQUENCE DESCRIPTION	ORGANISM
Q969F8	KISSR_HUMAN KiSS-1 receptor	*Homo sapiens*
Q924U1	KISSR_RAT KiSS-1 receptor	*Rattus norvegicus*
F1R5R4	KISS1 receptor a danio	*Danio rerio*
F1QZU4	KISS1 receptor b danio	*Danio rerio*

Table.2 Amino acid composition of KISS receptor

Amino acids	Q924U1	Q969F8	F1R5R4	F1QZU4
Alanine A	14.6	16.6	6.0	6.3
Arginine R	7.1	7.8	4.9	4.4
Asparagine N	2.8	2.8	4.1	4.4
Aspartic acid D	2.0	2.0	2.7	2.2
Cysteine C	3.8	3.8	2.7	4.1
Glutamine Q	3.3	2.3	2.7	5.2
Glutamic acid E	1.3	1.3	3.8	2.5
Glycine G	5.6	6.3	4.1	2.5
Histidine H	3.8	2.5	2.2	1.6
Isoleucine I	1.8	1.5	8.5	6.6
Leucine L	13.1	13.8	8.2	11.3
Lysine K	1.0	1.3	4.6	2.2
Methionine M	1.8	1.8	3.3	4.4
Phenylalanine F	4.0	3.8	8.5	5.5
Proline P	8.6	9.0	4.4	4.4
Serine S	7.1	6.8	9.0	7.7
Threonine T	4.8	3.5	6.0	8.2
Tryptophan W	2.3	2.0	1.9	1.6
Tyrosine Y	3.0	3.3	4.6	5.5
VALINE V	8.3	8.0	7.7	9.3

Table.3 Physicochemical properties of AFPs from different fish varieties are computed using Expasy's ProtParam tool

Accession number	Length	Molecular Weight	Isoelectric point(PI)	Negative charged Residue	Positive charged Residue	Extinction coefficient	Instability index	Aliphatic index	GRAVY
Q924U1	396	42889.14	9.70	13	32	67380	43.29	96.92	0.318
Q969F8	398	42586.04	9.93	13	36	64245	48.25	99.67	0.383
F1R5R4	366	42052.21	9.14	24	35	64455	34.23	93.20	0.271
F1QZU4	364	41578.00	8.67	17	24	63675	45.12	103.05	0.449

Table.4 Transmembrane prediction results by TMHMM

Accession number	TM1	TM2	TM3	TM4	TM5	TM6	TM7
Q969F8	44-66	79-101	121-138	158-180	204-226	264-286	306-328
Q924U1	44-66	79-101	121-138	158-180	204-226	261-283	Nil
F1R5R4	40-62	75-97	112-131	152-174	202-224	260-282	297-319
F1QZU4	46-68	89-111	124-146	158-180	208-230	271-293	308-330

Table.5 Secondary structure prediction by GOR server in percentage

Accession Number	Alpha helix	Extended	Beta turn	Random coil
Q924U1	28.79	18.43	0.00	52.78
Q969F8	34.92	15.08	0.00	50.00
F1R5R4	25.68	28.69	0.00	45.63
F1QZU4	15.11	36.54	0.00	48.35

Table.6 Secondary structure prediction by SOPMA server in percentage

Accession Number	Alpha helix	Extended	Beta turn	Random coil
Q924U1	41.92	14.39	2.53	41.16
Q969F8	42.46	13.82	4.02	39.70
F1R5R4	41.26	15.57	2.46	40.71
F1QZU4	47.80	15.93	2.20	34.07

The very high aliphatic index infers that these receptor may be eurythermal means having a wide range of temperature tolerance (Table 3). The Grand Average Hydropathy (GRAVY) is a phenomenon used for calculating the hydropathy value of all the amino acids upon the number of residues in the sequences. Fish kisspeptn receptor proteins analysed in this study having a range from 0.271 to 0.449. We also performed trans-membrane (TM) region identification with the help of TMHMM Server v. 2.0.

This helps in the identification of various trans-membrane helices with their corresponding length and it helps to distinguish between membrane proteins from stable proteins. All are seven trans-membrane helices but theoretically Q924U1 showing six trans-membrane which is not acceptable (Table 4).

The KISS peptin receptor is seven trans-membrane G protein coupled receptor reported by several researchers. The secondary structures of AFPs were predicted by SOPMA (Table 5) and GOR (Table 6).

Ramchandarn plot validation of is done and allowed and disallowed region is calculated. Most of the amino acid found in the allowed region. For the study the receptor-ligand interaction the identification of ligand binding sites is an important parameter and there are no individual methods that can provide the optimal prediction for all proteins (Kumar *et al.*, 2018).COACH server (Jianyi *et al.*, 2013) has used to identify the active binding site of this receptor.

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