International Journal of Research and Review
Vol. 10; Issue: 5; May 2023
Website: www.ijrrjournal.com
Research Paper *E-ISSN: 2349-9788; P-ISSN: 2454-2237*

Structural Prediction of Melanocortin Receptor (MC4R) of *Carassius Auratus*: An *in-silico* Approach

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DOI: https://doi.org/10.52403/ijrr.20230530

ABSTRACT

Carassius auratus commonly known as goldfish is one of most attractive fish used as ornamental fishes throughout the world. From the ancient times, the melanocortin system is considered to be conserved ranging from teleosts to mammals. Various researches in several fish species presented melanocortin receptors 4 (MC4R) is involved in different functions in fish body. Therefore, in-silico analysis of MC4R of Carassius auratus has been carried out in the study to know the physicochemical properties and 3D structural confirmation of the protein. Sequence containing accession number XM_026207258.1 was taken from National Center for Biotechnology Information (NCBI) and then processed to Expasy's protparam for the physicochemical characterization, SOPMA for secondary structure prediction. Template search was done by SWISSMODEL/Workspace and Swiss-Pdb-Viewer was used for prediction of 3D structure of the concerned protein. The results suggested MC4R is a stable, hydrophobic and slightly basic nature of protein. The secondary structure of the analysed MC4R protein suggested presence of 50.77% alpha helix, 19.69% extended strands and 3.38% beta turns along with 26.15% random coils. Moreover, it might be resolved from the Ramachandran plot that the structural prediction of MC4R is correct in prediction. Further, predicted 3D structure of MC4R protein can also be utilized for docking and simulation studies in future for finding out its relative binding affinity and functional action mechanism aspects. Moreover, the study is an approach for reducing the sequence data and solved structures gap by X-ray crystallography and NMR spectroscopy, which are also tedious and expensive laboratory techniques in application as well.

Keywords: Melanocortin receptors 4 (MC4R); *In silico* analysis; *3D* structural prediction; *Carassius auratus.*

INTRODUCTION

Carassius auratus commonly known as gold fish is one of most attractive fish used as ornamental fishes throughout the world. The raising world demand has opened the market for ornamental fishes possessing with unique shapes or colours following the use of transgenic technologies. By the use genes encoding fluorescent proteins through transgenic technologies, production of variable colours in fishes and a variety of combinations can be achieved[1]. Goldfish is a freshwater fish in the family of Cyprinidae of order Cypriniformes. High demand of gold fish as aquarium fish exists, which made it as a best choice for culture among ornamental fish farmers.

From teleosts to mammals, the melanocortin system is considered to be conserved. Five subtypes of melanocortin receptors, ranging from type 1 to type 5 (MC1R-MC5R), are available. These melanocortin receptors are G protein receptors (GPCRs) consisting of seven transmembrane domains[2-4]. Recently, studies on melanocortin receptors 4 (MC4R) have extensively been done by various researchers in several fish species. The first cloned MC4R was from zebra fish[5]. There-after, various researchers presented more than twenty fish MC4R, which are available in the database of NCBI along with

describing the molecular mechanism of melanocyte stimulating hormone binding to MC4R[6,7].

Various documented reports suggested that MC4R is engaged in energy regulation and body weight maintenance[6,8]. But, the MC4R expression was observed in various parts of the mammalian body such as: in CNS[9,10] and peripheral tissues like: heart, lung, kidney, renal nerve, ureter, intercostal muscle of skull bone[11] and enteroendocrine L cells[12] etc. The MC4R is also widely expressed in a variety of fish tissues. Expression of MC4R in goldfish was observed in gill, spleen, retina and ovary[13]. Other reports on flounder, MC4R was mainly presented its expression in liver, ovary, and testis[5,14]. MC4R was also found to be available and expressed in well manner in the brain, pituitary, and gonads of spotted scats, both in male and female[15]. Further, investigation on MC4R of common carp showed increased expression in brain, testis, eye, pituitary and heart[16]. Recently, grass carp presented greatly expressed MC4R in the brain and eye, but muscle, heart, intestine, liver, gill, spleen, and kidney presented low expression level[17]. The widespread expression pattern of MC4R in various fishes suggested that it might be involved in different physiological actions in different manner in a tissue specific way.

MC4R is involved in several physiological mechanisms in mammalian and non-mammalian animal models. MC4R is well documented in regulating mammalian energy homeostasis[4], maturity-onset obesity[18] or monogenic obesity[10,19-22]. Very recent documentation also presented that regulation of energy homeostasis by the MC4R is also functional for lower vertebrates including fishes. A study on goldfish presented that NDP-MSH or MTII injection inhibits the intake of food, but blocking by HS024 antagonist to MC4R raises that food intake, which ultimately concluded as MC4R is exhibiting a tonic inhibitory activity on food intake[13, 23]. MTII injection (ICV) of also reduces food intake in case of rainbow trout, but HS024 and SHU9119 antagonist injection effects on MC3/4R which ultimately increases food intake[24]. In cavefishes, a MC4R non-synonymous mutation contributes to boosted appetite, growth, and resistance to starvation [25]. Other studies indicate the MC4R involvement in modulation of reproductive function via affecting the reproductive hormones secretion, and subsequently effects sexual maturation in fishes[4]. Gonadal MC4R expression of was observed in goldfish[23], Ya-fish[26], and snakeskin gourami[27]. Documentation also suggested that MC4R replicas located on the sex chromosomes participate in the onset of sexual maturity and modulation in both male and female platyfish and swordtails[28, 29]. Hence, still now very limited information exist about the functional role of fish MC4R. Involvement of MC4R in several other physiological activities, like: body colour mediation, blood glucose modulation and homeostasis etc. Therefore, understanding of structural composition by *in-silico* approach has been considered hereby in the study for fish melanocortin receptor, MC4R which has potential role and importance. Specifically, MC4R is involved in several multidimensional physiological activities of fish body. To understand structural aspects of MC4R, *Carassius auratus* sequence was considered for the study, which also considered as an economically significant species for the aquaculture industry treating as an ornamental fish.

MATERIALS AND METHODS

The amino acid sequence of melanocortin receptor (MC4R) of *Carassius auratus* was retrieved from National Center for Biotechnology Information (NCBI) heaving the accession number XM_026207258.1. Expasy's protparam server was used for analysis of physicochemical properties including molecular weight, theoretical pI, % total number of negative and positive residue, the composition of amino acids, instability

index, grand average of hydropathicity (GRAVY). The secondary structure characteristics of the protein was observed by Self-Optimized Prediction Methods with Alignment (SOPMA). SWISSMODEL/Workspace was used for template selection searches. For homology modeling, template 7f53.1.E along with sequence identity of 75.53% was selected. Finally, by using Swiss-Pdb-Viewer, the 3-dimensional structure of the protein was predicted. Then, by using RAMPAGE server, the predicted structure was validated.

RESULTS AND DISCUSSION:

Goldfish (*Carassius auratus*) breeding was occurred about 1000 years ago in the Song dynasty of China [30]. The red skin colour was its fixed first trait from ancestral time [30]. But, this ornamental fish was artificially selected in an extreme intensive manner from wild grey crucian carp (*C. auratus*) during its domestication history[30,31]. Moreover, 70 genetically and 180 variant strains are available showing differences in their eye and body shape, coloration, scales, fins, and hood morphology[32]. Such wide morphometric diversity exhibited goldfish as an exceptional model to study vertebrate development and evolution[31,32]. A high quality reference genome data has been assembled for a common goldfish strain[33]. Very recently, Gan et al., (2021) demonstrated over the transcriptomic analysis in goldfish to improve genome annotation for unravelling skin pigmentation[34]. Structural bioinformatics is a useful area to fulfil gap which lies in between the sequence data to *3D* structural confirmation of MC4R of *Carassius auratus.*

Prediction of physicochemical properties:

The analysed physicochemical properties of MC4R protein by Expasy's protParam server presented 325 amino acid containing polypeptide along with estimated molecular weight 36385.52 kDa and theoretical isoelectric point (pI) is 8.60 which presents slightly basic nature of the protein. Maximum presence of Leucine (12.6%) and minimum presence of Aspartic acid and Tryptophan (1.5%) amino acid was observed in the linear structure of the MC4R protein molecule (Table.1). The total numbers of positive and negatively charged residues of MC4R are (Arg + Lys): 20 and (Asp + Glu): 14, respectively. The estimated instability index (ll) of the MC4R protein is 37.44 which specifies the protein as stable. Aliphatic index of the MC4R protein measures 117.32 which represents its thermostability along with the relative volume occupied by aliphatic side chains. The positive value (0.744) of the grand average of hydropathicity (GRAVY) demarcates that MC4R is a hydrophobic protein.

Amino acid composition	Numbers of Amino acid	% of Amino acids
Ala (A)	24	7.4%
Arg (R)	12	3.7%
Asn(N)	16	4.9%
Asp (D)	5	1.5%
Cys (C)	15	4.6%
Gln (Q)	8	2.5%
Glu (E)	9	2.8%
Gly (G)	16	4.9%
His (H)	9	2.8%
Ile (I)	35	10.8%
Leu (L)	41	12.6%
Lys (K)	8	2.5%
Met (M)	18	5.5%
Phe (F)	17	5.2%
Pro (P)	9	2.8%
Ser (S)	27	8.3%
Thr (T)	18	5.5%
Trp (W)	5	1.5%
Tyr (Y)	12	3.7%
Val (V)	21	6.5%

Table 1: Amino acid composition of MC4R

Secondary structure prediction:

By using SOPMA, the secondary structure of MC4R was predicted keeping default parameters of window width 17, similarity threshold 8 and number of states 4 of the server as standards[35]. MC4R secondary structure prediction suggested presence of alpha helix (50.77%) with 165, extended strands (19.69%) with 64 numbers, Beta turn (3.38%) with 11 and Random coil (26.15%) with 85 (Figure 1).

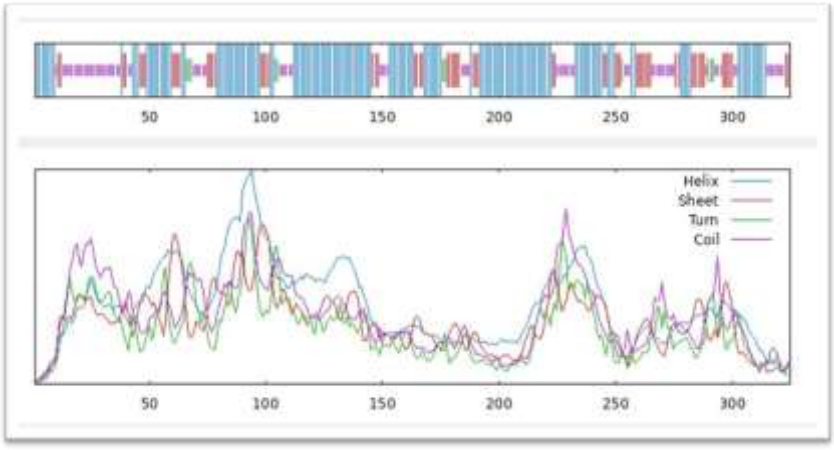

Figure 1: Secondary structure of MC4R by SOPMA

***3D* structure prediction by Homology modeling and validation of the model:**

For homology modeling, template 7f53.1.E along with sequence identity of 75.53% was selected for MC4R by using SWISSMODEL/ Workspacec (Figure 2). By using Swiss-PdbViewer, the *3D* structure of MC4R was predicted on the basis of homology modeling [36]. QMEAN score assessment determines the model quality (Figure 3) via the composite scoring method. Several standard decoy sets including a molecular dynamics simulation decoy was utilized for assessment of QMEAN representing statistically significant models and discriminate them good from bad models[37]. Validation by Ramachandran plot (phi/psi) is a significant parameter to understand the predicted *3D* structure (Figure 4) of the proteins was correct or not (Figure 5). The stereochemical analysis[38] of MC4R by RAMPAGE server presented blue lines representing helix, red lines representing strand and green representing turn and loops. The lines in the plot designate the preferred areas. The outer lines encircle the area within which 90% of all crosses of the same colour were observed; the inner lines indicate the 50% area.

Figure 2: Template selection by SWISSMODEL/ Workspace for MC4R homology modelling

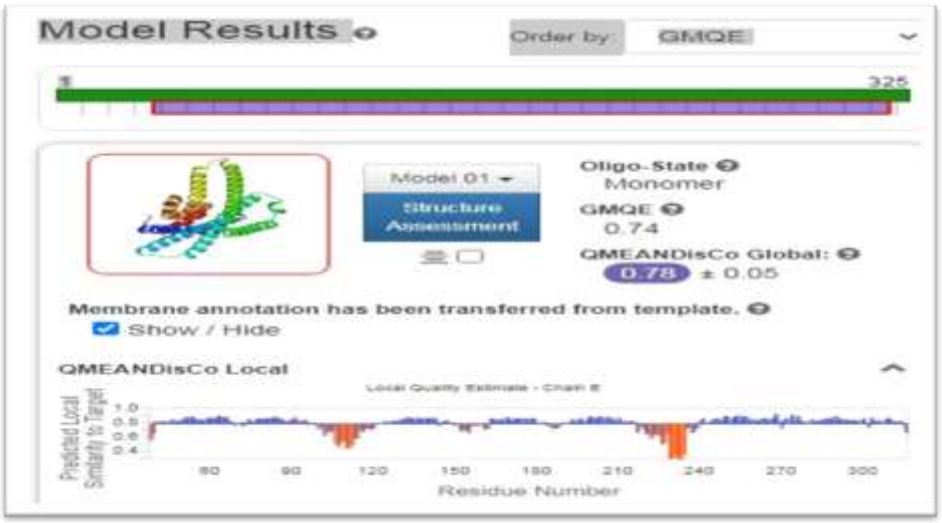

Figure 3: Model quality of MC4R estimated by QMEAN score

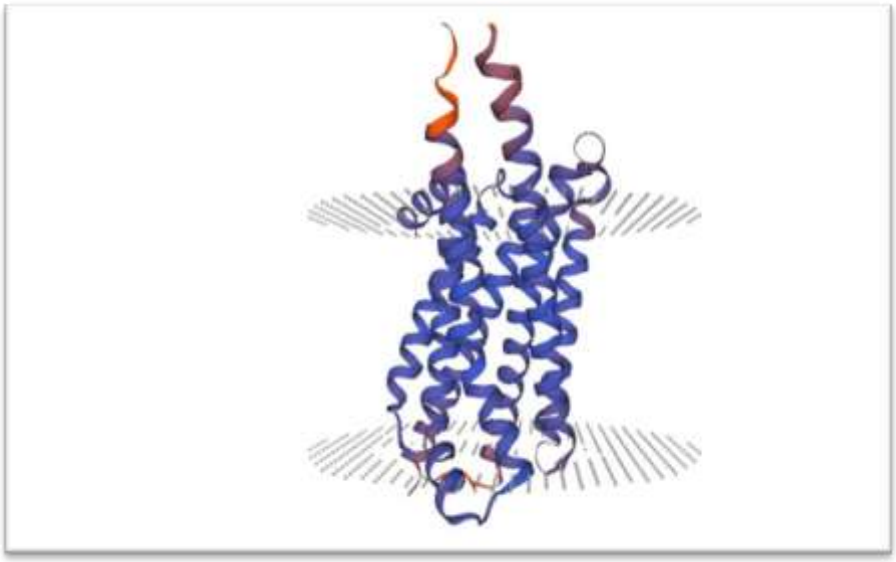

Figure 4: Predicted 3D structure of MC4R

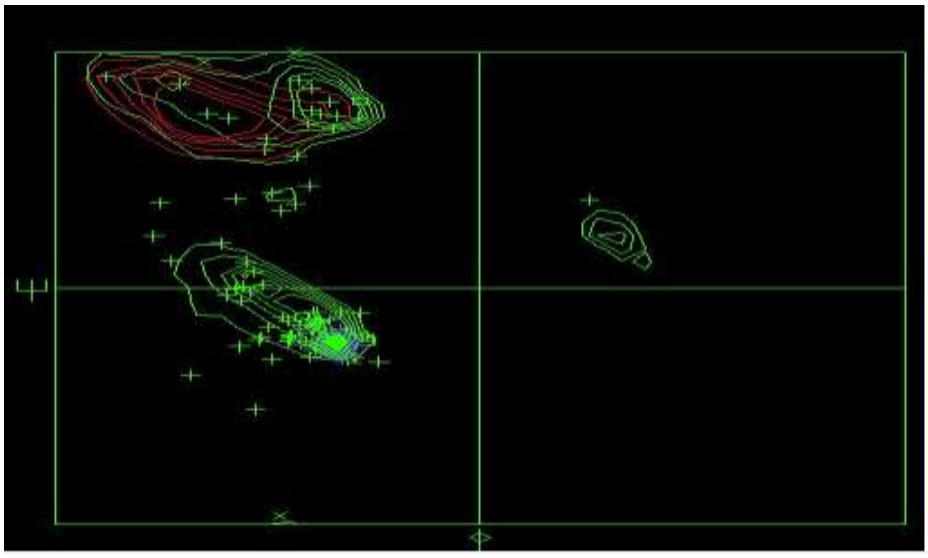

Figure 5: Validation of predicted 3D structure of MC4R by Ramachandran plot

CONCLUSION

Various structural and physicochemical parameters of MC4R protein were analysed and presented by this study. The predicted *3D* structure emphasized a conceptual direction about the receptor protein MC4R of *Carassius auratus*, which might help to understand the interaction of the concerned protein involved in the various physiological activities of the fishes. Although, NMR spectroscopy and X-ray crystallography are most convenient practical method of *3D* structure prediction, but involves huge time, monetary support and labour in implication. But, application of such *in-silico* bioinformatics tools and servers minimizes the gap generated by the available sequence data and solved structures. Based on present findings, it could be concluded that the MC4R is a stable, hydrophobic and slightly basic nature of protein. The secondary structure of the analysed proteins suggested presence of 50.77% alpha helix, 19.69% extended strands, 3.38% beta turn along with 26.15% random coils. Moreover, it might be resolved that predicted *3D* structure is expected to be accurate in prediction by observing the Ramachandran plot of MC4R. Further, the *3D* structural confirmation of MC4R protein can also be utilized for docking and simulation studies in future for finding out its relative binding affinity and functional action mechanism aspects.

Declaration by Authors

Acknowledgement: Author is thankful to the Principal, Rammohan College, Kolkata, West Bengal for encouragement and moral support.

Source of Funding: None

Conflict of Interest: The authors declare no conflict of interest.

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How to cite this article: Samik Acharjee. Structural prediction of melanocortin receptor (MC4R) of *Carassius auratus*: an *in-silico* approach. *International Journal of Research and Review*. 2023; 10(5): 244-251. DOI: *https://doi.org/10.52403/ijrr.20230530*

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