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In *silico* analysis of freshwater fish major histocompatibility complex class II alpha

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Abstract

Major histocompatibility complex (MHC) plays important roles in the immune system of vertebrates. This current study aimed to clearly understand the properties and structures of the MHC class II alpha (MHC IIα) proteins selected from freshwater fish species using *in silico* analysis. The MHC IIα of *Ctenopharyngodon idella*, *Oreochromis niloticus*, *Cyprinus carpio*, *Danio rerio*, *Oncorhynchus mykiss*, and *Ictalurus punctatus* were used**.** The molecular weight of MHC IIα of fish species was arranged from 17,054.5 to 26,358.9 Da. The three domains: "Class II histocompatibility antigen, alpha domain" (MHC_II_alpha), "Immunoglobulin C-Type" (IGc1) and "Transmembrane region" were found in the proteins. Physicochemical characterisation showed theoretical isoelectric point (pI: 4.31~5.3), total number of positive and negative residues (+R/-R: 19~32/13~20), extinction coefficient (EC: 18,910~29,910/19,410~30,410 $M^{-1}.cm^{-1}$, assuming that all pairs of cysteine residues form cysteines/all cysteines are reduced), instability index (II: 27.1~41.4), aliphatic index (AI: 73.3~93.3) and Grand average of hydropathicity (GRAVY: -0.240~0.156). Cysteine residues and disulphide bonds were determined from the proteins. In secondary structure prediction, excepting for the protein of common carp (extended strand was dominated), all proteins were composed of random coils as predominant, followed by extended strands, alpha helix and beta turn. Three dimensional structures of proteins were predicted performing SWISS-MODEL server. All models were evaluated being accepted and reliable based on structural evaluation and stereochemical analyses. This study provides knowledge of the physiochemical characterisations, structure features and functions of MHC IIα from freshwater fishes that is useful for further researches on the field of immune-related study of aquatic animals in future.

Keywords: Freshwater fish, MHC IIα, physicochemical characterisation, protein structure.

1. Introduction

Major histocompatibility complex (MHC) was known as a set of genomic regions or genes that encodes major histocompatibility antigens and plays key roles in the immunity of all vertebrates [1 - 3]. MHCs recognise and bind peptides from invasive agents such as pathogens for presenting to specific immune cells in the immune response mechanisms of animals [4]. MHC molecules directly interact with molecules of pathogens that are presented to have evolved adaptively, it is likely that much of these variation results from positive selection on the recognising and presenting pathogens abilities [5]. The MHC is essential in studying for disease resistance, mate choice, sexual selection and adaptive molecular evolution in vertebrates [5]. Basically, MHC gene is composed of MHC I and MHC II classes. Among these, MHC I is divided into classical (Iα) and non-classical (Iβ) groups based on their structures, functions and expression patterns; and MHC II comprises the α and β chains [1 & 6]. The peptide-binding sites of both MHC classes under three-dimensional (3-D) structures are mostly homologous [7]. While the MHC class I molecules presents peptides of endogenously synthesised proteins to CD8+ T cells, the MHC II functions in presenting processed exogenous antigens to CD4+ T cells [1, 8 & 9]. MHC II is found in many immune-related cells, including macrophages, lymphocytes and dendritic cells, that stimulate

immune responses from other cells [5 & 10]. Previously, several MHC II genes have been cloned and characterised from fish species, mostly are from ray-finned fish [1, 5, 11 - 15].

In silico approaches, known as performing computational tools via computer simulation, plays a crucial role in molecular biological studies, providing better comprehensive on the structural and functional properties of a protein of organisms. In silico analysis using algorithms, online servers and software through computer simulation are now available for utilisation in analysing of genomic, proteomic and evolutional data. These computational tools are useful in changing the raw sequence of proteins and nucleic acid into analytical and relative information [16]. In fact, the physicochemical characteristics, molecular functions and structural features of a protein may be predicted and analysed through using in silico analysis. To date, the properties and structures of proteins applying in silico analysis has been widely carried out in a broad of organisms such as bacteria [17], plants [18], mammals [19], and fish species [20 - 25]. However, computational studies on characterisation of structures and functions of MHC II proteins of fish species are still limited. Hence, the efforts on studying the physicochemical characteristics and homology modelling of MHC IIα proteins of some worldwide cultured freshwater fishes using in silico analyses were undertaken in this study. This study results provide information on the physicochemical characteristics, structural features, and molecular functions of MHC IIα proteins in different freshwater fish species which are useful for applying in the field of immune-related study of aquatic animals.

2. Materials and Methods

2.1. Protein retrieval

Six MHC IIα protein sequences (in FASTA format) of freshwater fish species, including grass carp (Ctenopharyngodon idella) (Accession No.: ABW37740.1), Nile tilapia (Oreochromis niloticus) (AEO44577.1), common carp (Cyprinus carpio) (CAA64708.1), zebra fish (Danio rerio) (AAA16369.1), rainbow trout (Oncorhynchus mykiss) (CAB96451.1) and channel catfish (Ictalurus punctatus) (AAD39868.1) produced from the NCBI (National Center for Biotechnology Information) database (http://www.ncbi.nlm.nih.gov/) were used for this study analyses.

2.2 Sequence alignment

Multiple sequence alignment between homologous MHC IIα sequences from different fish species was performed using Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/). Neighbour-joining phylogenetic analysis of protein sequences was generated using MEGA 6.0 [26], and the topological stability of the trees was evaluated by 1000 bootstrap replications.

2.3. Physicochemical and functional characterisation

The physicochemical characterisations: theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction coefficient (EC), instability index (II), aliphatic index (AI) and grand average hydropathy (GRAVY) of investigated proteins was analysed by searching on the Expasy's ProtParam server (http://web.expasy.org/protparam/). The positions of cysteine residues, number of cysteine presenting in pairs along the protein sequences and the presence of predicted disulphide bonds were computed using CYS_REC (http://linux1.softberry.com/). The domain structures of proteins were predicted employing Simple Molecular Architecture Research Tool (SMART) (http://smart.embl-heidelberg.de/).

2.4. Protein structure prediction

Secondary structure of proteins was predicted using SOPMA server (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html), with the default parameters (window width: 17, similarity threshold: 8, and number of states: 4). The 3-D structures of the MHC IIα proteins were predicted preforming homology modelling server, SWISS-MODEL (http://swissmodel.expasy.org/). The modelled structure was picked up based on the sequence identity to its templates [27]. The stereochemical quality and accuracy of the predicted models were analysed using PROCHECK's Ramchandran plot analysis [28 & 29]. The best selected models were based on the total number of residues in the most favoured regions, additional allowed region, generously allowed region and disallowed region as well as an overall G-factor value. A good quality model would be expected to have over 90% in the most favoured region and a cut-off value (>-0.5) of overall G-factor [29]. The selected models were then evaluated using ProQ [30] and ProSA [31], each of which validated protein models based on different validation parameters.

3. Results and Discussion

3.1. Sequence analysis and alignment

Sequence analysis results revealed that the domains "Class II histocompatibility antigen, alpha domain" (MHC_II_alpha) consisting of 79~81 amino acids, the "Immunoglobulin C-Type" (IGc1) comprising 71~72 amino acids, and the "Transmembrane region" containing 23 amino acids were found in all MHC IIα protein sequences (Figure 1).

A multiple amino acid sequence alignment of proteins was performed (Figure 2A). The result indicated a high amino acid sequence similarity between the MHC IIα homologs of six studied fish species. A neighbour-joining phylogenetic tree was constructed using MEGA 6.0 (Figure 2B). Two major sequence clusters, Nile tilapia and rainbow trout were in single cluster with other fish species were recorded through phylogenetic analysis, indicating the different monophyletic groups of taxa, with the close relationship from cyprinids.

3.2. Physicochemical and functional characterisation

The physicochemical characterisations of proteins were obtained analysing Expasy's ProtParam (Table 1). The values of isoelectric point (pI) of proteins were ranged from 4.31 to 5.3 (less than 7), implying the acidic character of these proteins. The pI values function in protein purification by isoelectric focusing on a polyacrylamide gel. Total number of negatively and positively charged residues (+R/-R) was ranged from 19 to 32 and 13 to 20, respectively. The extinction coefficient (EC) of proteins measured at 280 nm was in a range of 18,910 to 29,910 M-1.cm-1 (assuming all pairs of cysteine residues form cysteines), and 19,410 to 30,410 M-1.cm-1 (assuming all cysteine residues are reduced). The high value of ECs in this study implied a high concentration of cysteine along the protein sequences, functioning in quantitate the protein concentration in a volume of solution. The instability index (II) value evaluates the stability of proteins in a test tube; it was recommended that a protein is stable when its II value is smaller than 40 and as unstable when such value is above 40.

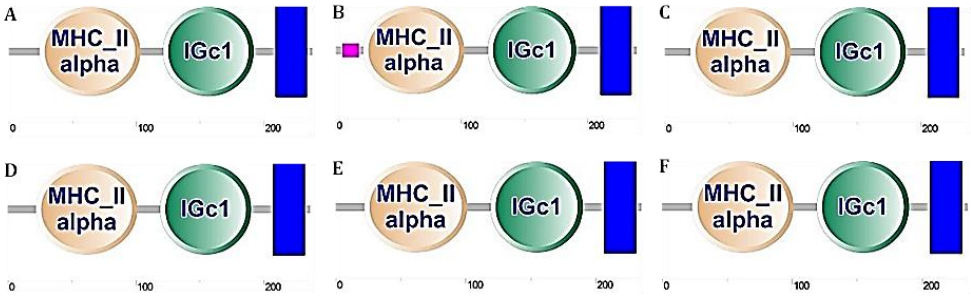

Figure 1 Structures of MHC IIα proteins of grass carp (A), Nile tilapia (B), common carp (C), zebra fish (D), rainbow trout (E), and channel catfish (F), generated by Simple Molecular Architecture Research Tool (SMART), showing "Class II histocompatibility antigen, alpha domain" (MHC_II_alpha), "Immunoglobulin C-Type" (IGc1) and "Transmembrane region" (blue colour boxes).

Table 1 Parameters computed using Expasy's ProtParam tool

Species	No. of aa	Mol. Wt.	pI	+R	-R	EC*	II	AI	GRAVY
Grass carp	238	26,358.9	4.92	20	29	21,805/21,430	27.1	82.6	-0.020
Nile tilapia	239	26,157.5	4.81	20	31	19,410/18,910	39.9	75.0	-0.233
Common carp	234	25,500.9	4.54	16	29	21,805/21,430	40.4	93.3	0.156
1 Zebra fish	236	26,205.3	4.31	14	34	23,295/22,920	38.0	78.0	-0.127
Rainbow trout	152	17,054.5	5.30	13	19	24,325/23,950	41.4	89.6	0.020
Channel catfish	235	26,328.6	4.51	15	32	30,410/29,910	37.1	73.3	-0.240

**First value is based on the assumption both cysteine form cysteines and the second assumes that both cysteine residues are reduced*

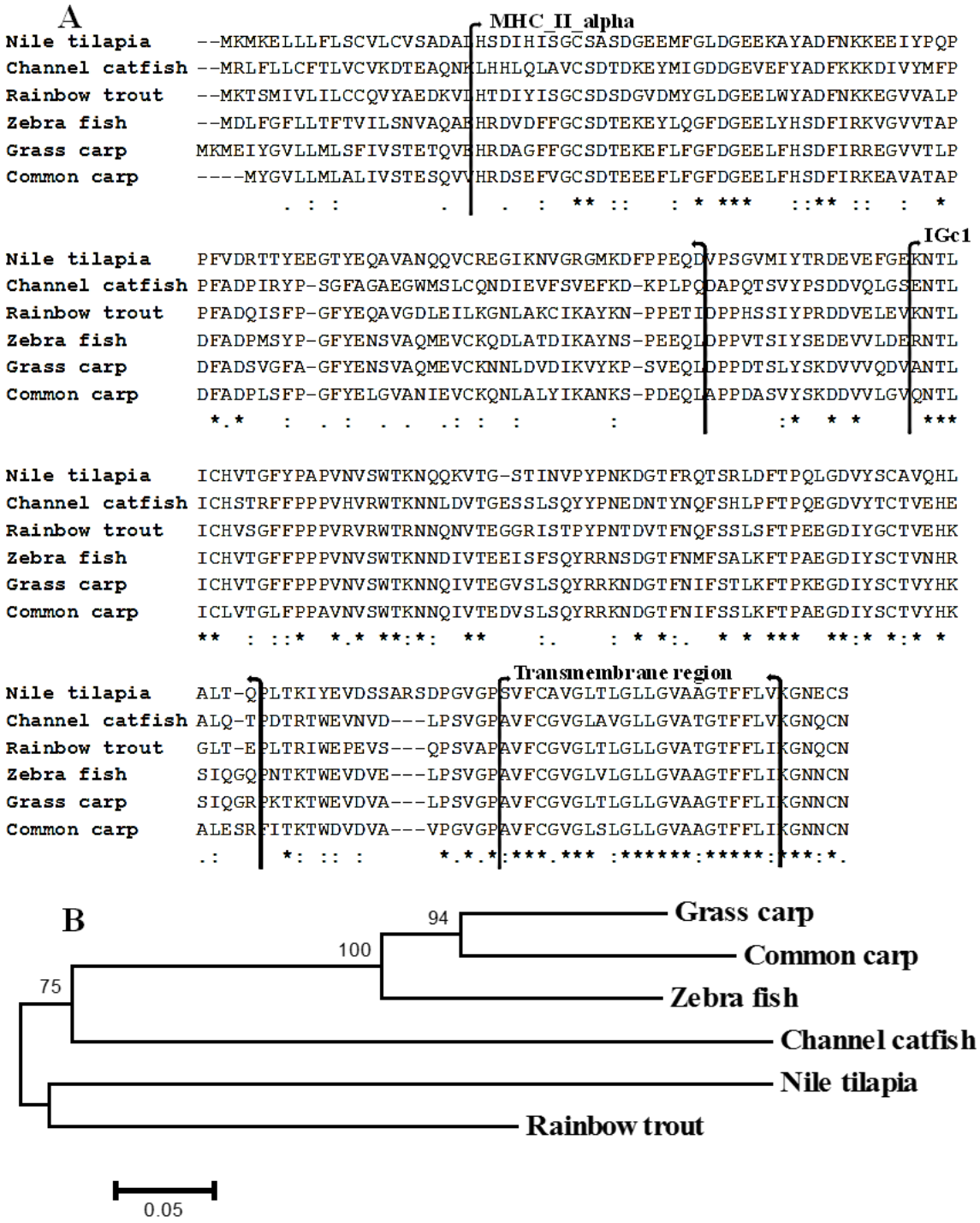

Figure 2 Sequence alignment and phylogenetic analyses between fish MHC IIα sequences. (A) Multiple sequence alignment. The asterisk marks (*), colon (:), dot (.) and dash (-) indicated identical amino acids, conserved substitutions, semi-conserved and deletions, respectively. The "Class II histocompatibility antigen, alpha domain" (MHC_II_alpha), "Immunoglobulin C-Type" (IGc1) and "Transmembrane region" were indicated by arrows. (B) A neighbour-joining phylogenetic tree showing the relationships between the MHC IIα homologs. The numbers at the branches indicated bootstrap values (1000 replications). The bar (0.05) indicated the genetic distance.

This study results showed that the II value of proteins was in a range of 27.1~41.4, showing the proteins of common carp and rainbow trout are unstable (II>40) and the rest are stable (II<40). The aliphatic index (AI) is regarded as a measure for the stability of globular proteins at high temperatures, associating with the mole fraction of aliphatic side chains (alanine, valine, isoleucine, and leucine) in the proteins. The AI values of protein in this current study was in a range of 73.3~93.3. The high AI values indicated that the proteins may be stable in extensive temperature ranges. Grand average hydropathy (GRAVY) of a protein is calculated by adding the hydropathy value for each amino acid and dividing by the total residues in the sequence. The GRAVY of proteins in this study

was ranged from -0.240 to 0.156. Herein, the low GRAVY values referred to the hydrophilic property and better solubility in water of the proteins. The amino acid composition in all retrieved protein sequences was found with a high proportion of valine, leucine and glycine in their polypeptide chains.

Disulphide bonds are significant in the protein folding and stability, which are generated between the thiol groups of cysteine residues by oxidative folding process. In this study, the cysteine residues in the proteins were determined using CYS_REC server. The results revealed that all proteins contain cysteine residues in their sequences; and the most probable patterns of pairs of cysteine were found (Table 2), suggesting that all proteins contain disulphide linkages in their sequences. These disulphide bonds may be significant in folding and stability of proteins in a fluctuating cellular environment [35].

3.3. Protein structure prediction

The secondary structures of MHC IIα proteins from different fish species were predicted using SOPMA server; the results were showed in Table 3. The results revealed that except for the MHC IIα protein of common carp (extended strand is dominant), all have a predominance of random coils and followed by extended strands, alpha helix and beta turn. Meanwhile, the rest of secondary structure elements were not found. As stated previously, the random coils are usually referred to the absence of regular secondary structure, suggesting the extended strand is the prevailing secondary structure feature of all proteins.

Undoubtedly, protein structure often reflects its functions. Indeed, 3-D structures provide better comprehension on the basically biological functions of proteins, which allow an effective design of experiments. In this study, the 3-D structures of MHC IIα proteins were performed applying a homology modelling server, SWISS-MODEL (Table 4). Based on sequence identity (28.4~35.8%, Table 4), three different templates available from the Protein Data Bank (PDB) were picked to build models: 1ktd.1.A for grass carp, common carp, zebra fish, and channel catfish; 1fng.1.A for Nile tilapia; and 3c60.1.C for rainbow trout. The resolution of proposed models was in a range of 1.9~3.1 Å. The searching analysis showed that the coverage residue of the models of grass carp, Nile tilapia, common carp, zebra fish, rainbow trout and channel catfish was in a range of Glu23~Asp201, Leu21~Ala200, Val19~Asp197, Glu21~Asp199, Leu21~Glu198, and Lys21~Asn198, respectively, comprising all domains of the proteins. The 3-D structures of predicted models were shown in Figure 3.

Table 2 Disulphide bond patterns of pairs predicted using CYS_REC tool

Species	CYS_REC
Gras carp	Cys32-Cys182
Nile tilapia	Cys126-Cys181
Common carp	Cys2-Cys3
Zebra fish	Cys30-Cys124
Rainbow trout	Cys30-Cys180
	Cys124-Cys210
Channel catfish	Cys124-Cys180

Table 3 Secondary structure elements of MHC IIα of fish species using SOPMA

Element	Grass carp	Nile tilapia	Common carp	Zebra fish	Rainbow trout	Channel catfish
Alpha helix	20.2	24.7	18.0	23.7	16.6	10.2
3_{10} helix	0.0	0.0	0.0	0.0	0.0	0.0
Pi helix	0.0	0.0	0.0	0.0	0.0	0.0
Beta bridge	0.0	0.0	0.0	0.0	0.0	0.0
Extended strand	35.7	25.1	39.3	30.9	31.9	38.3
Beta turn	8.0	11.3	5.1	9.8	11.5	10.6
Bend region	0.0	0.0	0.0	0.0	0.0	0.0
Random coil	36.1	38.9	37.6	35.6	40.0	40.9
Ambiguous states	0.0	0.0	0.0	0.0	0.0	0.0
Other states	0.0	0.0	0.0	0.0	0.0	0.0

Table 4 Homology modelling of three-dimensional (3-D) structures of MHC IIα of fish species using SWISS-MODEL

Index	Grass carp	Nile tilapia	Common carp	Zebra fish	Rainbow trout	Channel catfish
Template	1ktd.1.A	1fng.1.A	1ktd.1.A	1ktd.1.A	3c60.1.C	1ktd.1.A
Resolution (Å)	2.4	1.9	2.4	2.4	3.1	2.4
Sequence identity (%)	35.8	31.5	35.8	31.8	35.8	28.4
Coverage range	Glu23-Asp201	Leu21-Ala200	Val19-Asp197	Glu21-Asp199	Leu21-Glu198	Lys21-Asn198
Ramachandran plot						
Total number of residues	179	180	179	179	178	178
Residues in most favoured regions (%)	92.3	88.8	89.3	93.6	86.3	92.2
Residues in additional allowed region (%)	7.7	10.5	8.8	6.4	13	7.2
Residues in generously allowed region (%)	0	0.7	1.9	0	0.7	0.7
Residues in the disallowed region (%)	0	0	0	0	0	0
G-factor	0.61	-0.31	-0.26	-0.25	-0.25	-0.3
ProQ						
Lgscore	1.173	0.829	1.553	1.284	1.267	1.313
MaxSub	0.146	0.087	0.162	0.177	0.119	0.16
ProSA						
Z-Score	-4.25	-4.24	-5.67	-5.69	-5.34	-4.67

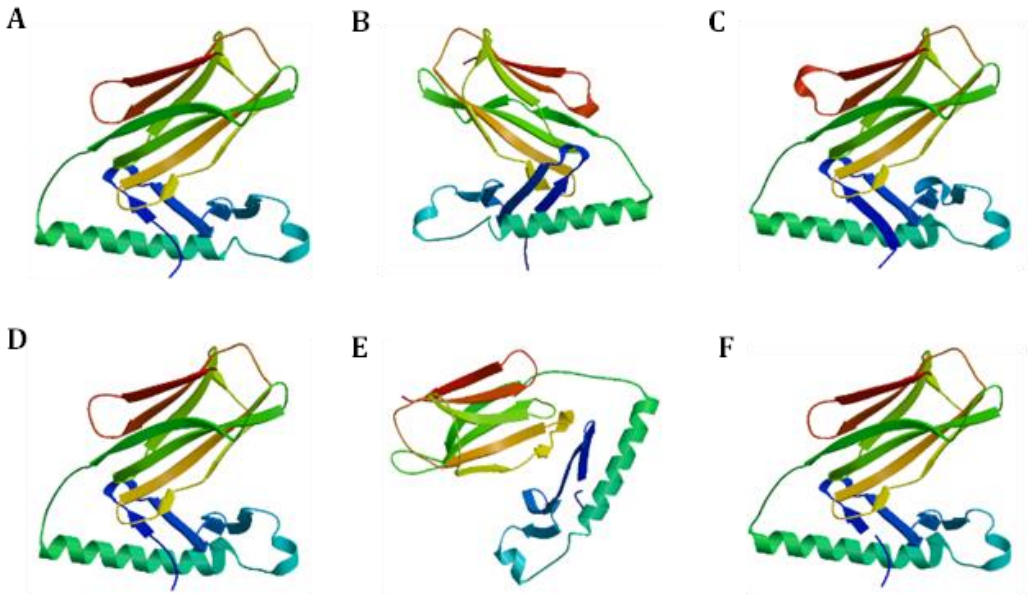

Figure 3 Three-dimensional structures of MHC IIα proteins from grass carp (A), Nile tilapia (B), common carp (C), zebra fish (D), rainbow trout (E), and channel catfish (F), rendered by SWISS-MODEL server.

The stereochemical quality and accuracy of proposed models were examined performing PROCHECK analysis (shown in Table 4). The analysis results revealed that the predicted models for MHC IIα of grass carp, zebra fish and channel catfish have over 90% (92.3%, 93.6% and 92.2%, respectively) of residues in the most favoured region, indicating that these homology models were good quality. The rest consisting of almost all of the residues (>90%) were found in the most favoured and additional allowed regions combined, implying acceptable models [29]. The overall average G-factor of dihedral angles and main-chain covalent forces was

ranged from -0.31 to 0.61 (>-0.5), suggesting good quality of the proposed models [29]. Lgscore and MaxSub values, ranging from 1.173 to 1.553 and 0.119 to 0.177, respectively, indicated correct quality of the models, excepting for the model of Nile tilapia (Lgscore: 0.829 and MaxSub: 0.087), suggesting unsuitable model (Table 4) [30]. The Z-Scores (analysed using ProSA server) of the models were ranged from -4.24 to -5.69 (Table 4), which were within the range of the typical scores for native proteins of the similar size; the plots of residue energy values were predominantly negative (data not shown), suggesting the proposed models were good quality [31] & [38]. Taken together, the validated results indicated that the predicted models for MHC IIα of fish species can be accepted and reliable. On the whole, these structures may be significant in functional analysis of experimentally derived crystal structures of proteins [39].

4. Conclusions

In this study, six MHC IIα proteins of freshwater fish species were selected to characterise using computational tools. Physicochemical and functional characterisations of the proteins were analysed. Secondary structure prediction revealed that except for the MHC IIα protein of common carp, all have a predominance of random coils among the secondary structure elements, followed by extended strands, alpha helix and beta turn. The 3-D structures of proteins were predicted and validated; the results suggested that all proposed models are reliable and valid. This study provided information on the physicochemical characteristics, structural properties and molecular functions of fish MHC IIα, which are useful for further studies on specific functions, e.g. immune responses against to pathogens, of this protein in aquatic animals.

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