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Freshwater Fish Myeloid Differentiation Primary Response Protein MyD88: Characterisation and Homology Modelling by Using Computational Tools

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Abstract

MyD88 is a mediator to trigger the downstream signal in Toll/Toll-like receptor pathway which plays an important role in the innate immune system. In this study, computational tools were used to analyse the properties and structures of freshwater fish MyD88 proteins. The MyD88 proteins from fish species, including *Cyprinus carpio* Linnaeus 1758, *Carassius carassius* (Linnaeus 1758), *Oreochromis niloticus* (Linnaeus 1758), *Danio rerio* (Hamilton 1822), and *Ictalurus punctatus* (Rafinesque 1818) were used in this study. Physicochemical characterisations were performed by computing molecular weight, theoretical isoelectric point, amino acid composition, total number of positive and negative residues, extinction coefficient, instability index, aliphatic index, and grand average of hydropathicity. Cysteine residues were found in all MyD88s, excepting channel catfish MyD88; disulphide bond pattern of pairs were predicted in common carp, crucian carp, Nile tilapia and zebrafish. Random coils were dominating among secondary elements, followed by helices and strands. Three dimensional structures of proteins were analysed and validated by using PROCHECK's Ramachandran plot, ProQ and ProSA. The results indicated that all predicted models may be used as structures for investigated fish MyD88s. This study provides basic knowledge of the properties, structures and functions of MyD88 proteins from freshwater fish species and is useful for further studies on specific functions of this protein.

Introduction

MyD88 plays an important role in innate immune system and is first identified as a myeloid differentiation primary response gene, which in M1 myeloleukemic cells induces response to interleukin-6 (Lord et al. 1990). MyD88 has a bi-partite structure composed of amino terminal death domain and carboxyl terminal Toll/Interleukine-1 receptor homology domain that mediates interaction with Toll-like receptors (TLR) and interleukine-1 receptor (IL-1R) (Wesche et al. 1997). MyD88 is known as an adaptor protein which triggers the downstream signal in Toll/TLR pathway, which subsequently activates the nuclear factor-kappa B through interleukin

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1 receptor-associated kinase and tumour necrosis factor receptor-associated factor 6 leading to the induction of pro-inflammatory cytokines (Medzhitov et al. 1998). This stimulation processing is a key component for the activation of innate immunity and host defence (Fuse et al. 2005). MyD88 has been identified and extensively studied in both vertebrates and invertebrates (Zhang et al. 2012). In fish, MyD88 has been defined and characterised in several species such as zebrafish *Daniorerio* (Hamilton 1822) (Meijer et al. 2004; van der Sar et al. 2006), Japanese flounder *Paralichthys olivaceus* (Temminck & Schlegel 1846) (Takano et al. 2006), large yellow croaker *Pseudosciaena crocea* (Richardson 1846) (Yao et al. 2009), rainbow trout *Oncorhynchus mykiss* (Walbaum 1792) (Rebl et al. 2009), Atlantic salmon *Salmo salar* Linnaeus 1758 (Skjaeveland et al. 2009), common carp *Cyprinus carpio* Linnaeus 1758 (Kongchum et al. 2011), and rock bream *Oplegnathus fasciatus* (Temminck & Schlegel 1844) (Whang et al. 2011).

Computational tools are essential in the fields of molecular biology, especially, in protein and peptide sciences, from the genome scale to the atomic level (Xu et al. 2000). Several computational tools such as software, algorithms and online servers are available and provide great methods for the characterisation and analysis of proteins. The physicochemical characteristics such as molecular weight (Mol. Wt.), chemical formula, theoretical isoelectric point (pI), extinction coefficient (EC), instability index (II), aliphatic index (AI), and grand average of hydropathicity (GRAVY) can be analysed easily by using computational tools. Besides, molecular functions and structures prediction for protein can be performed from protein sequences. Recently, the application of computational tools as an entrance of understanding the properties and structures of proteins, comprising antifreeze proteins (Hossain 2012) and mannose binding lectin homologue proteins (Goel et al. 2013) have been carried out from fish species. This present study describes the application of computational tools to predict the properties and homology modelling of three dimensional structures for MyD88 proteins from freshwater fish species, which will provide a better understanding of physicochemical characteristics, structural features and the molecular functions of this protein from different fish species.

Materials and Methods

Protein sequence retrieval

MyD88 protein sequences of five different freshwater fish species were retrieved from the NCBI (National Center for Biotechnology Information) database (http://www.ncbi.nlm.nih.gov/) in the FASTA format and used for further analysis in this study. The basic information of MyD88 proteins is shown in Table 1.

Table 1. Freshwater fish MyD88 proteins retrieved from NCBI database.

Common name	Scientific name	Accession No.	No. of aa
Common carp	*Cyprinus carpio* Linnaeus 1758	ADQ08685.1	285
Crucian carp	*Carassius carassius* (Linnaeus 1758)	AGY96960.1	284
Nile tilapia	*Oreochromis niloticus* (Linnaeus 1758)	AHN82524.1	288
Zebrafish	*Danio rerio* (Hamilton 1822)	NP_997979.2	284
Channel catfish	*Ictalurus punctatus* (Rafinesque 1818)	NP_001187207.1	279

Physicochemical characterisation

Physicochemical characterisation, including Mol. Wt., pI, amino acid composition, total number of positive (+R) and negative residues (-R), EC (Gill and Von Hippel 1989), II (Guruprasad et al. 1990), AI (Ikai 1980) and GRAVY (Kyte and Doolottle 1982) of MyD88 proteins were analysed using the Expasy's ProtParam prediction server (Gasteiger et al. 2005).

Functional characterisation

The types of protein (membrane and soluble) of selected sequences were identified using SOSUI server (Hirokawa et al. 1998). The CYS_REC (http://linux1.softberry.com/) was used to predict the presence of disulphide bonds and their bonding patterns, which are crucial in defining the functional linkage and the stability of a protein. CYS_REC determines the positions of cysteines and total number of cysteines, presenting in pairs, along the protein sequences. NCBI Conserved Domain Database (NCBI CDD) (Marchler-Bauer and Bryant 2004) was used to search for the conserved domains (CDs) within a protein or coding nucleotide sequences in the target proteins.

Protein structure prediction

POLYVIEW-2D server (Porollo et al. 2004) was used in this study to predict the secondary structure of MyD88 protein sequences from different fish species.

Homology modelling was used to generate a reliable three dimensional structure of the MyD88 protein sequences by employing SWISS-MODEL server (Schwede et al. 2003; Arnold et al. 2006). The stereo chemical quality and accuracy of the predicted models were analysed and evaluated by using Ramachandran plot analysis (Ramachandran et al. 1963) with the PROCHECK program (Laskowski et al. 1996). The structural model analysis was represented by Swiss PDB Viewer (Guex and Manuel 1997). The best selected models were based on criteria of overall G-factor, number of residues in the favoured, allowed, generously allowed and disallowed regions. A good quality model would be expected to have over 90% in the most favoured region. The selected models of three dimensional structures were then evaluated using online servers, ProQ (Cristobal et al. 2001) and ProSA (Sippl 1993; Wiederstein and Sippl 2007), each of which validates protein models based on different validation parameters.

Results

Physicochemical characterisation

The MyD88 proteins from five freshwater fish species were retrieved from NCBI database in FASTA format and used for further analysis (Table 1). Parameters of physicochemical characterisation of the proteins, including Mol. Wt., pI, +R, -R, EC, II, AI and GRAVY were computed by employing Expasy's ProtParam server; the results were shown in Table 2.

The amino acid composition of retrieved proteins was illustrated in Table 3. Leucine was found in the highest amount, ranging from 9.8 to 10.8% in the MyD88 of the fishes (Table 3).

Table 2. Parameters computed using Expasy's ProtParam tool.

Fish species	Mol. Wt.	pI	-R	+R	EC*	II	AI	GRAVY
Common carp	33208.4	5.67	43	40	44515-43890	29.4	85.5	-0.295
Crucian carp	32967.2	5.52	42	39	44640-43890	36.2	88.6	-0.236
Nile tilapia	32888.1	5.05	44	36	41660-40910	42.3	89.7	-0.149
Zebrafish	32860.0	5.79	42	39	40170-39420	38.1	87.9	-0.236
Channel catfish	32423.3	5.31	41	35	44515-43890	43.1	89.4	-0.275

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

Table 3. Amino acid composition (in %) of MyD88 proteins from freshwater fish species computed using Expasy's ProtParam tool.

Amino acid	Common carp	Crucian carp	Nile tilapia	Zebrafish	Channel catfish
Alanine	5.3	6.0	6.6	5.6	5.4
Arginine	6.0	6.3	5.6	6.3	6.5
Asparagine	2.5	2.8	2.1	2.1	2.9
Aspartic acid	7.7	7.7	8.3	8.1	7.5
Cysteine	3.9	4.6	4.5	4.6	3.9
Glutamine	3.2	3.2	2.4	3.5	4.7
Glutamic acid	7.4	7.0	6.9	6.7	7.2
Glycine	1.8	2.1	2.4	2.1	2.5
Histidine	0.7	0.4	0.7	1.1	1.1
Isoleucine	5.3	5.6	4.9	5.3	6.1
Leucine	9.8	10.6	10.8	10.6	10.4
Lysine	8.1	7.4	6.9	7.4	6.1
Methionine	2.5	2.1	3.1	2.1	2.2
Phenylalanine	4.9	4.6	3.8	4.9	4.3
Proline	6.0	5.6	6.6	5.6	5.0
Serine	5.3	4.2	5.9	5.3	6.1
Threonine	7.0	7.4	5.9	7.0	5.7
Tryptophan	1.8	1.8	1.7	1.8	1.8
Tyrosine	3.9	3.9	3.1	2.8	3.9
Valine	7.4	6.7	7.6	7.0	6.8
Pyrrolysine	0.0	0.0	0.0	0.0	0.0
Selenocysteine	0.0	0.0	0.0	0.0	0.0

Functional characterisation

The SOSUI server was used to distinguish between membrane and soluble protein from the protein sequences. Results showed that all MyD88 proteins from investigated fish species are soluble protein. Except for MyD88 from channel catfish all proteins contain cysteine residues, implying that disulphide linkages may be present in these proteins. The most probable pattern of pairs of cysteine residues form is presented in Table 4.

Table 4. Disulphide bond pattern of pairs predicted by using CYS_REC tool.

Species	Disulphide bond pattern
Common carp	Cys105- Cys156
	Cys221- Cys232
Crucian carp	Cys105- Cys268
	Cys125- Cys232
	Cys154- Cys221
Nile tilapia	Cys65- Cys111
	Cys225- Cys272
Zebrafish	Cys105- Cys268
	Cys125- Cys154

CDs identified in MyD88 from different fish species are shown in Table 5. All MyD88 contain CDs in their sequences. Two domain hits namely 'death domain of myeloid differentiation primary response protein' (Death_MyD88) and 'toll-interleukin 1- resistance' (TIR) were found in all MyD88 proteins.

Table 5. CDs found in MyD88 proteins by using NCBI CDD.

Species	Name of hit	Description	Interval of hit
Common carp	Death_MyD88	Death domain of MyD88	21-98
	TIR	Toll-interleukin 1- resistance	148-279
Crucian carp	Death_MyD88	Death domain of MyD88	21-98
	TIR	Toll-interleukin 1- resistance	148-284
Nile tilapia	Death_MyD88	Death domain of MyD88	22-100
	TIR	Toll-interleukin 1- resistance	152-288
Zebrafish	Death_MyD88	Death domain of MyD88	21-98
	TIR	Toll-interleukin 1- resistance	148-284
Channel catfish	Death_MyD88	Death domain of MyD88	23-100
	TIR	Toll-interleukin 1- resistance	143-279

Secondary structure prediction

The POLYVIEW-2D server performed the secondary structure prediction of MyD88 proteins. The results with three secondary structure elements, helix, strand, and random coil composing of target proteins are represented in Table 6. Results showed that random coils are outweighed among secondary structure elements, followed by helices and strands for all MyD88 proteins from fishes. Figure 1 generated by POLYVIEW-2D illustrates the secondary structure features of MyD88 from common carp as an example which includes residue numeration, amino acid sequence, graphical representation and three elements of secondary structure (helix, strand or bridges, random coils), confidence level for secondary structure prediction, and relative solvent accessibility.

Table 6. Calculated secondary structure elements (in %) by using POLYVIEW-2D.

Fish species	Helix	Strand	Coil
Common carp	42.5	9.82	47.7
Crucian carp	46.1	10.6	43.3
Nile tilapia	43.4	9.72	46.9
Zebrafish	44.7	9.51	45.8
Channel catfish	45.2	9.32	45.5

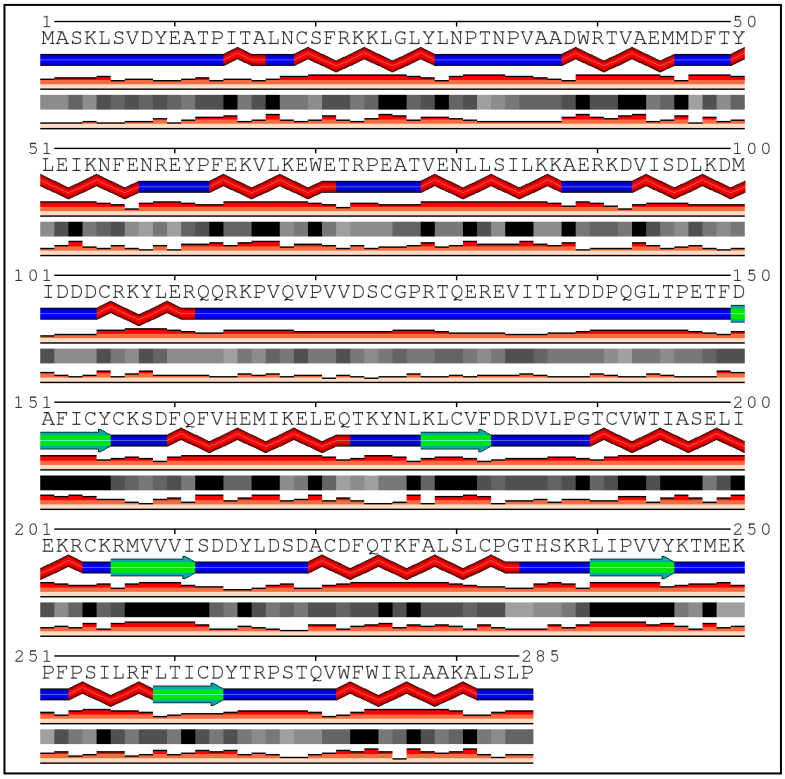

Fig. 1. Secondary structure of MyD88 protein from common carp as predicted by using POLYVIEW-2D server. H-alpha and other helices (view 1); H-alpha and other helices (view 2); E-beta-strand or bridge; C-coil; 0123456789 relative solvent accessibility (RSA) (0-completely buried (0-9% RSA), 9-fully exposed (90-100% RSA)); 0123456789 confidence level of prediction (0-the lowest level, 9-the highest level).

Homology modelling and model validation

This work resulted in four models with predictions based on the templates with PDB ID of 4dom (Snyder et al. 2013), 4eo7 (Snyder et al. 2013), 2js7 (Rossi et al. unpubl. data), and 2z5v (Ohnishi et al. 2009), showing the high sequence identity between the models and templates (Table 7). Three dimensional structures of predicted models are shown in Fig.2. Besides, the stereo chemical quality and accuracy of predicted models were performed using PROCHECK's Ramachandran plot analysis (Table 7). Results revealed that all selected models have a high number of residues in most favoured regions, ranging from 75.2 to 93.8. Moreover, the overall average G-factor of predicted models for MyD88 from different fish species ranged from -0.11 to 0.35 (Table 7). In addition, two servers of ProQ and ProSA were used to further validate the plausibility of these predicted models and the final validation results are shown in Table 8.

Table 7. Homology modelling of three dimensional models for MyD88 proteins from fish species by using SWISS-MODEL server.

Components	Common carp	Crucian carp	Nile tilapia	Zebrafish	Channel catfish
Template	4dom	4eo7	2js7	4dom	2z5v
Sequence identity (%)	78.4	79.1	76.7	79.9	80.3
Most favoured regions (%)	93.1	92.2	75.7	93.8	75.2
Additional allowed regions (%)	6.90	7.80	21.3	6.20	24.0
Generously allowed regions (%)	0.00	0.00	2.20	0.00	0.80
Disallowed regions (%)	0.00	0.00	0.70	0.00	0.00
Overall average of G-factor	0.15	0.35	0.00	0.20	-0.11

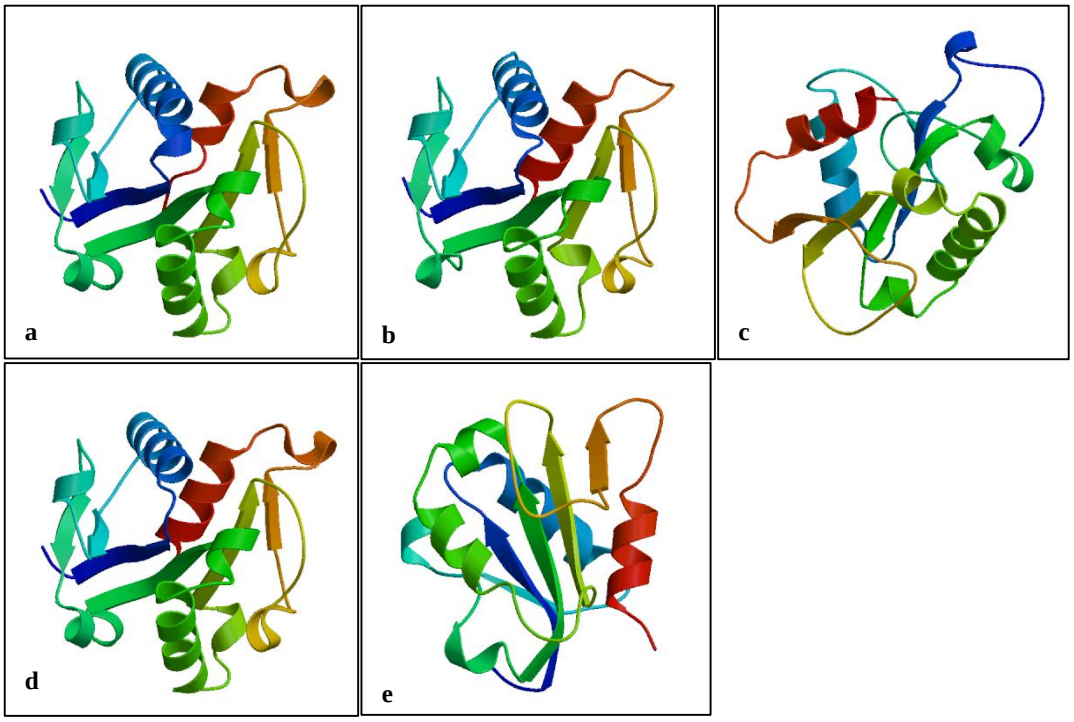

Fig. 2 Three dimensional structures of predicted models for MyD88 proteins by using SWISS-MODEL. (a) common carp, (b) crucian carp, (c) Nile tilapia, (d) zebrafish, and (e) channel catfish.

Table 8. Validation of predicted models by using ProQ and ProSA servers.

Species	ProQ		ProSA
	Lgscore	MaxSub	Z-Score
Common carp	7.061	0.913	-7.20
Crucian carp	6.602	0.841	-7.68
Nile tilapia	5.494	0.682	-7.58
Zebrafish	6.746	0.866	-7.58
Channel catfish	5.740	0.752	-6.68

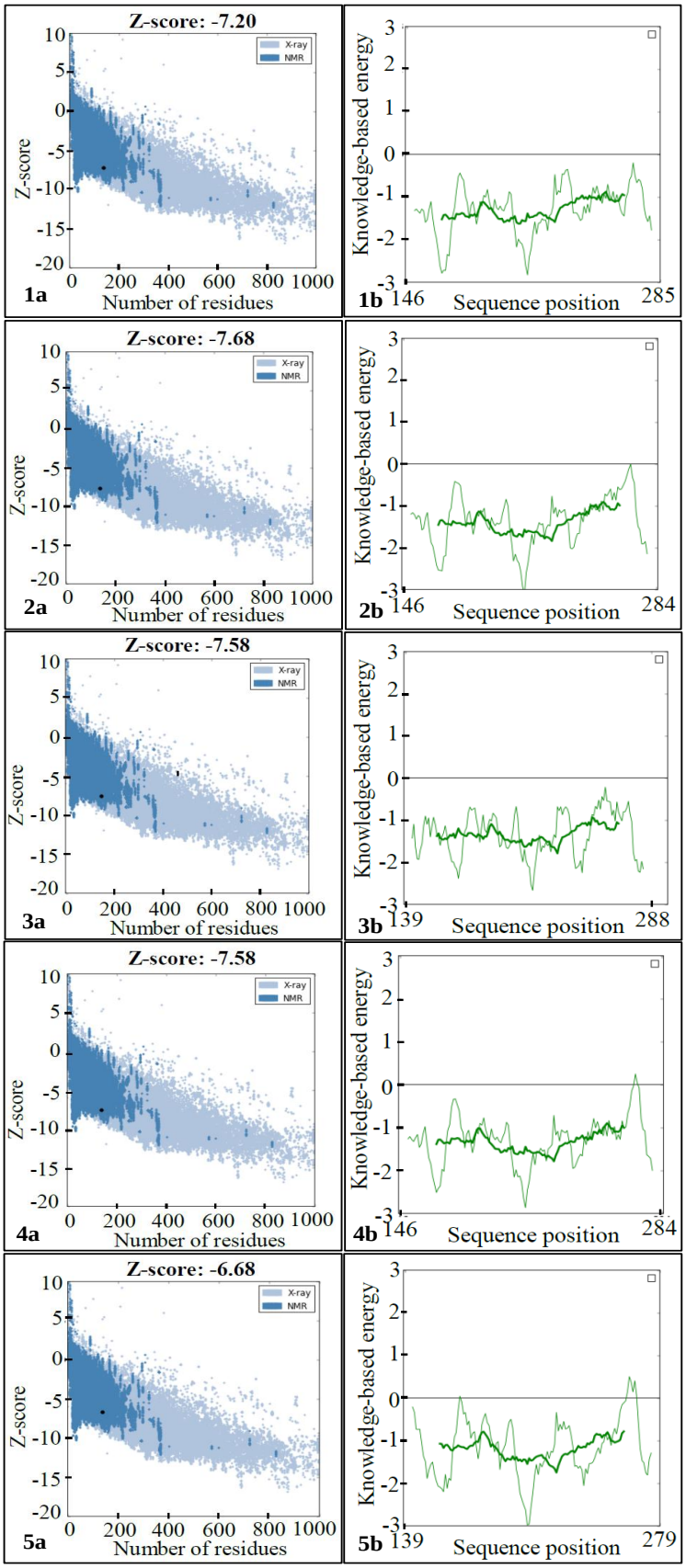

Fig. 3. ProSA-web server analysis of validation for predicted models for MyD88 proteins with Z-score (a) and energy plot (b) of (1) common carp, (2) crucian carp, (3) Nile tilapia, (4) zebrafish, and (5) channel catfish.

Discussion

Primary structure analysis of MyD88 proteins from different freshwater fish species was performed and then physicochemical characterisation was computed by employing Expasy's ProtParam server (Table 2). The pI value is the pH at which the net charge of protein is zero. The pI value can affect the solubility of a molecule at a given pH, but proteins are stable and compact at pI. Our work resulted in pI values ranging from 5.05 to 5.79 which were less than 7, indicating the proteins are acidic in character. These calculated pI values may be used for purification of the proteins on a polyacrylamide gel by isoelectric focusing. The EC of MyD88 proteins was measured at 280 nm. EC of proteins ranged from 40,170 to 44,640 $M^{-1}{\cdot}cm^{-1}$ (with the assumption that all pairs of cysteine residues come from cysteines), and from 39,420 to 43,890 $M^{-1}{\cdot}cm^{-1}$ (with the assumption that all cysteine residues are reduced). The high EC values imply a high concentration of cysteine, tryptophan and tyrosine in the protein sequences, which function to quantitate the protein-protein and protein-ligand interactions in solution. The II value is a measure to evaluate the stability of proteins in a test tube. Based on the fact that the occurrence of certain dipeptides significantly differs between stable and unstable proteins, a weight value of instability is assigned to each of the dipeptides. Using these values, it is possible to compute an II value for a given amino acid sequence. A protein is generally considered to be stable when II value is below 40 and unstable when II is above 40 (Guruprasad et al. 1990). Herein, the computed II values for MyD88 proteins ranged from 29.4 to 43.1. These results showed that except for MyD88 from Nile tilapia and channel catfish all are stable proteins (II <40). The AI is regarded as a positive factor for the increase of thermal stability of globular proteins which is directly related to the mole fraction of aliphatic side chains (alanine, isoleucine, leucine, and valine) in the protein (Ikai 1980). In this study, the obtained AI values of MyD88 proteins were high (from 85.5 to 89.7). The very high value of AI inferred that these proteins are stable for a wide range of temperature. The GRAVY value for a peptide or protein is calculated as the sum of hydropathic values of all the amino acids, divided by the number of residues in the sequence. The GRAVY of MyD88 proteins ranged from -0.295 to -0.149. This low value of GRAVY implies that these proteins are hydrophilic and soluble in natural conditions.

The searching analysis from NCBI CDD showed the two domains of Death_MyD88 and TIR present in all MyD88 proteins. These domains in MyD88s were in accordance with those found in other fish species, frog and mammals (Yan et al. 2012). This result indicates that all investigated MyD88 proteins have similar functions to such protein in other organisms. The death domain superfamily is important in the death signal transduction, the regulation of apoptosis and the inflammatory response (Weber and Vincenz 2001). The TIR domain plays crucial roles in activating the innate immune response by the TLR/IL-1R superfamily mediated pathway (Janssens and Beyaert 2003).

Protein structure often reflects its functions (Banerjee et al. 2012). In fact, the secondary structure of protein is known as the patterns of hydrogen bonds of backbone amino acids and carboxyl groups. The secondary structure of protein has been predicted based on empirical correlations between the frequencies of certain vibrational modes and types of secondary structure of polypeptide chains such as α-helix, β-sheet, β-turn, and random coil (Cai and Singh

1999). In our works, the POLYVIEW-2D server was used to predict whether a given amino acid lies in a helix, strand or coil. Results revealed that random coils are dominating among secondary structure elements, followed by helices and strands in all MyD88 from fish species. However, the random coils usually referred to the conformations that indicate an absence of regular secondary structure (Cai and Singh 1999). Thus, in the case of freshwater fish MyD88 proteins in this current study, helix is the prevailing secondary structure feature.

The modelling of the three dimensional structures of the protein in this study was performed by using the SWISS-MODEL server to obtain the best models for MyD88 proteins. Quality and reliability of models were checked by structure assessments, including Ramachandran plots, G-factor value, ProQ and ProSA. In Ramachandran plot analysis, three models for MyD88 from common carp, crucian carp and zebrafish comprised over 90% of residues number in the favoured regions, indicating that these models are good in quality (Ramachandran et al. 1963). The other two models showed the residue numbers lower than 90% (with 75.7% and 75.2% in Nile tilapia and channel catfish, respectively) which fell in the most favoured regions, but a higher residue number was calculated in the additional allowed regions; this suggests that these may be good models. Otherwise, the G-factor is an important index to evaluate the quality of stereo chemical property of proteins. In this work, the overall average G-factor of predicted models for MyD88 from different fish species ranged from -0.11 to 0.35, which are higher than the acceptable value (a cut-off value of -0.5), inferring that these are all good quality predicted models.

ProQ server used two quality measures (Lgscore and MaxSub) to further evaluate the quality of predicted structured models (Table 8). The Lgscore index resulted in a range of 5.494 to 7.061, fulfilling the criteria of the extremely good quality in all predicted models. The MaxSub index computed ranging from 0.682 to 0.913, indicating all predicted models are from very good to extremely good quality (Cristobal et al. 2001).

ProSA-web analysis results are shown in Table 8 and Fig. 3. ProSA is displayed by two quality measures, Z-score and a plot of its residue energies. The Z-score of predicted models was from -7.68 to -6.68, which were inside the range characteristic for native proteins (Figs. 3-1a, 2a, 3a, 4a and 5a), implying that the predicted models have properties as native structures. Moreover, the plot of its residue energies yielded negative values (Figs. 3-1b, 2b, 3b, 4b and 5b). This indicated that all predicted models are highly reliable structures and of good quality (Wiederstein and Sippl 2007).

Homology modelling on human MyD88 has been previously implemented in multiple studies. TIR domain structure of human MyD88 (at position 157-296) has been predicted by Ohnishi et al. (2009), and they reported that the human MyD88 TIR domain composed a central 5-stranded parallel β-sheet (βA–βE) surrounded by 4 α-helices (αA–αC and αE) in the secondary structure. MyD88 TIR domain shared the highest sequence similarity with toll-like receptor 2 structures when it was compared with other available structures (Ohnishi et al. 2009). Furthermore, based on structural modelling analysis, the role of two human MYD88 variants (S34Y and R98C) in the assembly of the Myddosome relating the death domain of MyD88,

IRAK4, and IRAK2 or IRAK1 was confirmed; this analysis also explained the imperfections observed in S34Y and R98C mutants (George et al. 2011). In addition to matrix protein of human metapneumovirus (Yadav et al. 2013) and human tyrosinase (Gupta et al. 2013), the authors used similar approaches for modelling and verifying the predicted protein structures. Comparatively, the results in this study suggest that the predicted models may be used as structures for MyD88 proteins from investigated fish species. To our knowledge, this study is the first time a protein MyD88 from freshwater fish was characterised and modelled by using computational methods.

Conclusion

In this study, MyD88 proteins selected from freshwater fish species were characterised by using computational tools. Physicochemical characterisations such as Mol. Wt., PI, +R, -R, EC, II, AI and GRAVY were analysed by using Expasy's ProtParam tool. Functional analysis recorded all MyD88 proteins are soluble proteins. For cysteine residues and disulphide linkages prediction, except channel catfish MyD88, all have cysteine residues presented in the protein sequences, inferring the presence of disulphide linkages in proteins. Secondary structure analysis revealed that MyD88 proteins contain a dominating number of random coils, followed by helices and strands. Three dimensional structures of MyD88 proteins were implemented by employing SWISS-MODEL. The results indicated that all predicted models may be used as structures for MyD88 from freshwater fish species. This study provides basic information on using computational tools to analyse the properties, structures and functions of MyD88 proteins which are useful for further studies on specific functions of this protein..

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