Full Length Research Article

Molecular Mechanics and Heavy Metal (Cd- Cadmium) Interaction on *Oreochromis mossambicus* (Tilapia) using Insilico Protocols

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Abstract

In this study, the effects of heavy metal (Cd- Cadmium) on the growth hormone (GH) protein of *Oreochromis mossambicus* (Tilapia) were analyzed. Molecular mechanics protocols are used to explore the 3D structure of the *Oreochromis mossambicus* and were visualized using advanced molecular visualization tools. The modelled structure was applied to a Metal protein binding server to identify the positions where the amino acids bind with the metal protein. The results clearly elucidated in a graphical manner which amino acid has a high binding affinity with the metal. The results were validated using an *Insilico* molecular visualization tool which clearly explains the binding positions of the 3D amino acids with Cd present in *Oreochromis mossambicus.* In conclusion, the molecular interaction between the growth hormone protein of tilapia was observed and it was found that the binding of Cd with the growth hormone inhibits the normal function of the growth of Tilapia. These results play a vital role in understanding the factors affecting the growth of Tilapia in the fish farming sector.

Key words: Protein modelling, Cadmium, Heavy metal binding prediction, Growth hormone protein, Tilapia

Tilapia is a native of Africa and the Middle East that has risen from obscurity to become one of the world's most productive and widely traded food fish [1]. Over the last three decades, there have been major advancements in tilapia farming around the world. They are farmed in around 85 countries throughout the world. Tilapia is a member of the Cichlidae family, which is part of the Perciformes order. There are around 70 species of tilapias, nine of which are exploited in global aquaculture [2].

Heavy metals are persistent contaminants in the aquatic environment that can cause serious sickness in fish, animals, and humans [3]. Lead (Pb) is more abundant in water bodies than cadmium (Cd), copper (Cu), chromium (Cr), manganese (Mn), and mercury (Hg), and is a growing global issue due to its negative effects on human health. It is a cumulative poison that is listed as one of the 129 priority pollutants by the Environmental Protection Agency [4]. Windblown dusts, forest fires, volcanic emissions, and sea salt sprays are the primary natural sources of Pb emissions.

Protein structure governs biophysical functions as well as interactions with other components such as metal ions, tiny ligands, and other proteins. Because metal ions can stabilize protein structures and catalyze reactions, discovering metal ion-binding sites is critical to understanding the biological significance of metal ion-binding proteins.

Genetic markers provide a dependable method for revealing genetic organization both within and between populations. Furthermore, genetic markers can help identify the species, individuals, or populations of origin of unknown samples, allowing authorities to monitor protected nature reserves. As a result, understanding the population genetic structure and genetic diversity of *Oreochromis niloticus* is critical for both conservation and fish breeding. Previous research looked at the genetic structure and diversity of Nile tilapia (*Oreochromis niloticus*) populations using phenotypic traits [5], allozymes [6] mitochondrial DNA [7] randomly amplified polymorphic DNA [8] or microsatellites [8], [9]. However, the genetic markers utilized to date have limited in terms of maximum resolution in recognizing the complex genetic structure found in Nile tilapia populations. Furthermore, no other study, to our knowledge, has attempted to investigate the efficacy of genetic markers in predicting the population of origin in putative unknown tilapia samples.

MATERIALS AND METHODS

Sequence retrieval system

The target protein sequence of the growth hormone (GH) of *Oreochromis mossambicus* (tilapia) was selected using NCBI genpept database in FASTA format.

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3D Molecular mechanics

The protein sequence of the growth hormone (GH) was converted into 3D structure using an automated protein homology modelling server called Swiss Model in order to predict the binding of the heavy metal. After modelling, the 3D structure was validated using ProCheck server for 3D structure quality assessment.

Protein- Heavy Metal binding prediction

The protein sequence of the growth hormone (GH) was analysed using a metal binding server in order to analyse binding affinities between the selected heavy metal and the GH protein of tilapia.

3D Structure Visualization

The modelled protein structure of the growth hormone which is bound to the heavy metal at the respective amino acid positions was viewed using advanced molecular visualization software, Discovery Studio.

RESULTS AND DISCUSSION

In this *Insilico* work, the growth hormone of tilapia fish, Oreochromis *mossambicus* (CAA72415.1) (tigH gene) was retrieved in FASTA format from NCBI GenPept database. The length of the gene sequence is 615 nt and the length of its protein sequence is 371 aa (Fig 1).

Metal binding prediction we use MIB server [10-11] in order to identify heavy metals binding amino acids positions. Essential metals play an important role in the normal metabolism of fish. However, their organs may also get accumulated with non-essential metals [13]. Fe, Cu, Zn and manganese (Mn) are examples of essential metals, and Hg, Pb, nickel (Ni) and Cd are a few examples of non-essential metals [14]. [13] reported that augmented concentrations of heavy metals in their body have a negative impact on the growth and development of fish at early life stages such as hatching, larval development and juvenile growth as they are highly sensitive to these metals during their early developmental stages than during adult stages. Obviously, fish establish a link for toxic heavy metals to get transferred from water to humans [15]. The deleterious impact of trace elements when taken beyond the recommended limit can be noxious (acute, chronic or sub-chronic), and heavy metals can be neurotoxic, carcinogenic, mutagenic or teratogenic. The habitual symptoms shown by humans with respect to metal [e.g., Cd, Pb, As, Hg, Zn, Cu and aluminium (Al)] poisoning comprise of convulsions, vomiting, ataxia, paralysis, gastrointestinal disorder, diarrhoea, hemoglobinuria, tremor, stomatitis, pneumonia and depression [16].

>CAA72415.1 tilapia growth hormone [*Oreochromis mossambicus*]
MNSVVLLLSVVCLGVSSQQITDSQRLFSIAVNRVTH
LHLLAQRLFSDFESSLQTEEQRQLNKIFLQDFCN
SDYIISPIDKHETQRSSVLKLLSISYGLVESWEFPSR
SLSGGSSLRNQISPRLSELKTGILLLIRANQDE
AENYPDTDTLQHAPYGNYYQSLGGNESLRQTYELL
ACFKKDMHKVETYLTVAKCRLSPEANCTL

The above amino acid sequence is that the growth hormone of Oreochromis mossambicus (Tilapia) retrieved using NCBI –Genpet database.
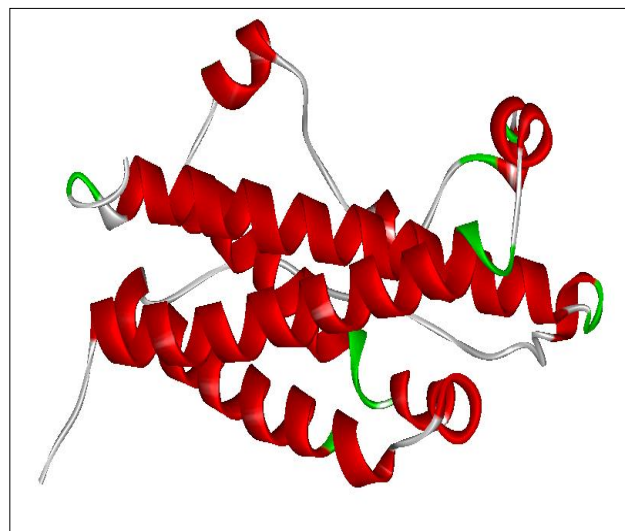
Fig 1 FASTA sequence of *Oreochromis mossambicus*

Fig 2 Protein modelling: 3D structure of the growth hormone of Tilapia

The above picture shows the 3D structure of the growth hormone of Tilapia in Solid Ribbon Model View, viewed using Discovery Studio Software.
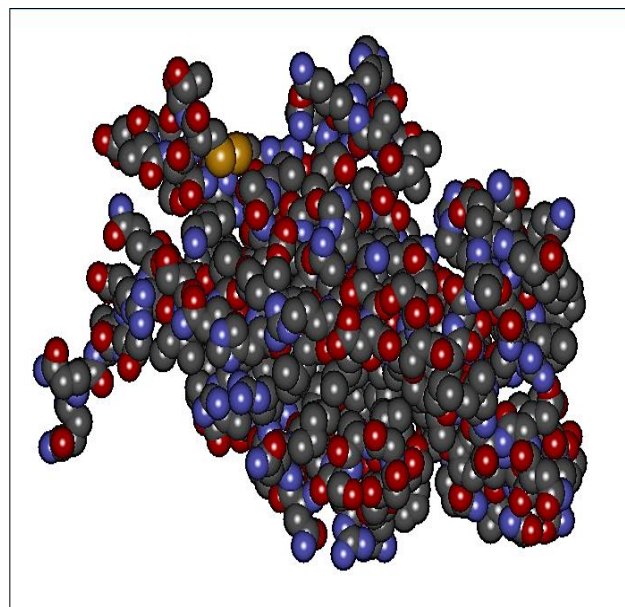

Fig 3 Protein modelling: 3D structure of the growth hormone of Tilapia

The above picture shows the 3D structure of the growth hormone of Tilapia in Space-filling model viewed using Discovery Studio Software.
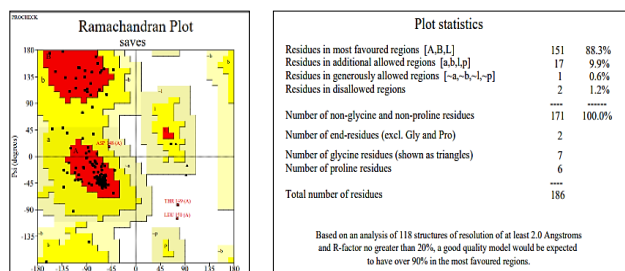

Fig 4 3D structure validation

The above picture gives details on the assessment of Ramachandran plot which shows the structural quality of the modelled protein.
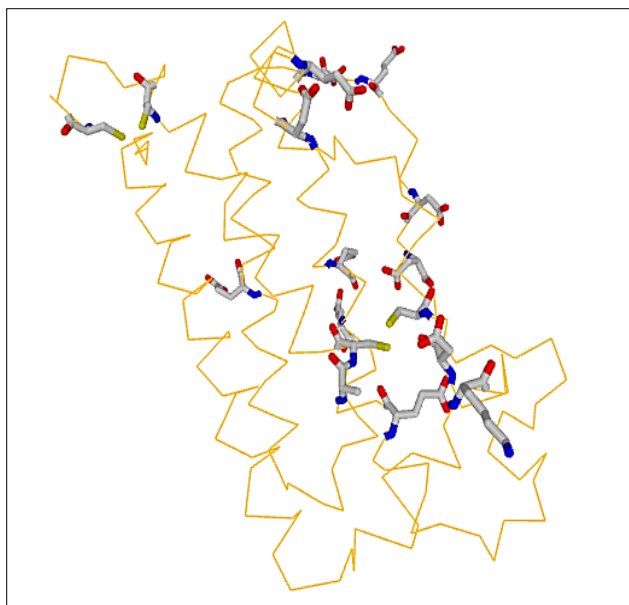
Fig 5 Heavy metal binding prediction

The above picture represents the 3D structure of the growth hormone of Tilapia in Stick model showing the various sites at which it is bound to the metal.
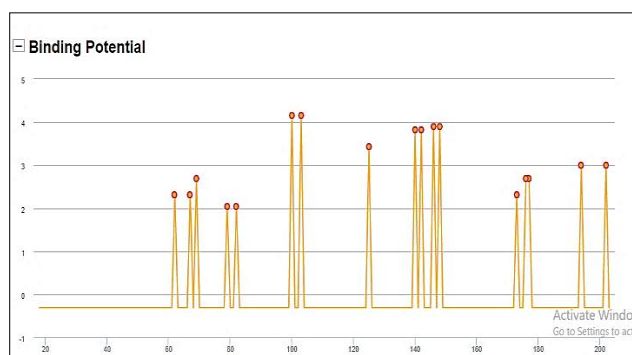

Fig 6 Heavy metal binding prediction

The above graphical picture shows the binding potential of the various amino acids of the growth hormone with the metal Cd.

Fig 7 3D heavy metal binding prediction

The above picture shows the 3D view of the growth hormone bound to Cd (gold colour) in Ribbon model and space-filling model respectively viewed using Discovery Studio Software.
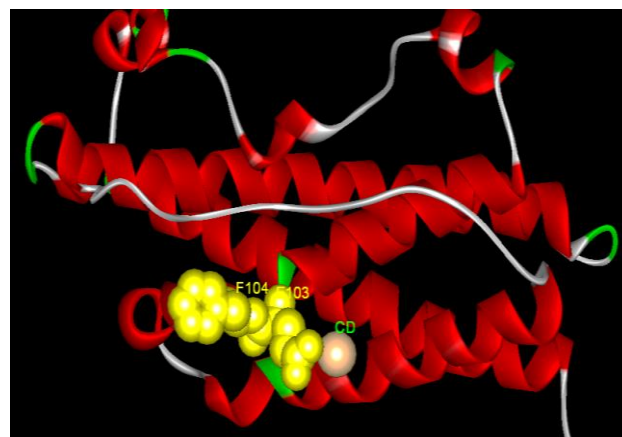

Fig 8 3D heavy metal binding prediction

The above picture shows the 3D view of the growth hormone bound to Cd (gold colour) in Ribbon model and space-filling model respectively also showing the respective amino acid positions in space-filling model viewed using Discovery Studio Software

Table 1 Metal binding summary

Target Species	Heavy Metal
Oreochromis mossambicus	
Phe (F) 100	Cd - Cadmium
Glu(E) 103	

The above table mentions that Phe (F) 100 and Glu (E) 103 are responsible for Cadmium heavy metal binding with the growth hormone of Tilapia.

In this research study, SWISS-MODEL was used to convert the amino acid sequence of (*Oreochromis mossambicus* –gH hormone protein) into 3D structure [Fig 1-]. SWISS-MODEL [17-20] was used to elaborately analyse the molecular and structural details of gH hormone protein for the purpose of docking. SWISS-MODEL is a server for automated comparative modelling of three-dimensional (3D) protein structures. Waterhouse *et al*. [18] computed models by the SWISS-MODEL server homology modelling pipeline which relies on ProMod3, an in-house comparative modelling engine based on Open Structure. The modelled 3D protein was completely evaluated using ProCheck server [21] for assessment of Ramachandran Plot. After modelling, the 3D structure of the mutated protein was validated using ProCheck server. (Fig 4) shows the assessment of Ramachandran Plot which confirms that there is no error (88.3 %) in the modelled protein. All the results obtained from our *in-silico* research study show that Cadmium binds with the potential amino acids (Phe (F) 100 and Glu (E) 103) (Table 1) of the growth hormone of *Oreochromis mossambicus* (Tilapia) (Fig 5-8). These amino acids are responsible for the absorption of metals into Tilapia fish thus affecting the growth hormone from performing its normal functions.

CONCLUSION

Previous studies on water pollution have detected the increase in the pollution of particular heavy metals in freshwater systems worldwide, especially in rivers. In this in silico study, we have identified the potential interacting amino acids involved in binding with non-essential metals like Cadmium (Cd) which inhibit the growth of Tilapia fish (*Oreochromis mossambicus*) in rivers. We conclude, that our study would play a vital role in fish farming industries to take

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preventive measures against water being polluted by non-essential metals to enhance to growth of fish.

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