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Full Length Research Article

Identification of Heavy Metal (Mercury (Hg)) Binding Regions in the Growth Hormone of *Oreochromis niloticus* Using In silico Protocols

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Abstract

Oreochromis niloticus or the Nile Tilapia is a cichlid fish which is an inhabitant of North Africa and the Levante area, including Israel, and Lebanon. In this In silico research investigation, two major findings are involved. We, first, analyze the protein sequence in the growth hormone of *Oreochromis niloticus* in order to identify the motif regions present in the sequence. Next, we predict the 3D structure of the protein sequence and then analyze the heavy metal binding sites, particularly, Mercury (Hg) present in it. In methodology, we use NCBI database to retrieve the protein sequence of the sps. in FASTA format. The retrieved sequence was analyzed for molecular functional details (motifs) using motif and domain servers. The 3D structure was explored using automated homology modelling servers and was applied into a metal-binding identification tool. Our results clearly explain that, in the protein sequence of the growth hormone of *Oreochromis niloticus*, the heavy metal, Mercury, binds with the functional part along with various other regions of the protein. Hence, we strongly conclude that the non-essential heavy metal, Mercury, is directly involved in the inhibition of the growth hormone of *Oreochromis niloticus*. The results obtained from our study play a vital role in rescuing aquatic species from the harmful effects of heavy metals.

***Key words*:** Mercury (Hg), *Oreochromis niloticus*, 3D structure prediction, Growth hormone

Tanzania is a tilapia diversity hotspot, with over 30 Oreochromis species, 10 of which are unique to the country [1]. *Oreochromis niloticus* is the most common tilapiine cichlid in Tanzania and around the world. Nile tilapia aquaculture in Tanzania has expanded from 958 MT in 2011 to 4080 MT in 2017, with a constant desire for additional expansion. Despite the enthusiasm and promise of tilapia aquaculture to contribute to local food supply, Tanzania currently lacks a selective breeding programme, a circumstance that is common in many African countries.

Heavy metals enter the aquatic food chain through two main mechanisms: direct intake of water and food through the digestive tract and non-dietary channels across permeable membranes such as the muscle and gills [2]. As a result, levels in fish often mirror levels present in the soil and water of the specific aquatic environment from which they are sourced [3], as well as the time of exposure [4]. Fish have the ability to collect heavy metals in their tissues at higher quantities than the environment due to absorption along the gill surface and the kidney, liver, and gut tract wall [4]. Heavy metal accumulation by organisms can be passive or selective, and differences in heavy metal accumulation by organisms can be due to differences in assimilation, egestion, or both [5]. Non-essential heavy metals such as Cadmium (Cd).

Mercury (Hg), and Lead (Pb) have no known essential role in living organisms; exhibit extreme toxicity even at very low (metal) exposure levels; and have been identified as the primary threats to all forms of life, particularly human health [6-7]. Toxic consequences arise when excretory, metabolic, storage, and detoxifying processes are no longer capable of counteracting absorption [8] resulting in physiological and histopathological alterations [2], [9-11]. Water physiochemistry can also influence these changes [4]. Heavy metals enter a fish's organs primarily through adsorption and absorption; the rate of accumulation is a function of uptake and depuration rates [4]. Non-essential metals, in addition to being poisonous and persistent, are bioaccumulated and internally controlled by several mechanisms such as active excretion and storage [12]. Significant differences in non-essential heavy metal levels have been observed between organs and fish species inhabiting the same freshwater body: Lake Balaton, Hungary [13], Iskenderun Bay, Turkey [14], and Three Gorges Reservoir, China [15]. Toxic heavy metal levels have been reported to be elevated in areas experiencing increased settlement, traffic, and agricultural activity [4-5]. Because fish is an important source

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of food for the general human population, the levels of non-essential trace elements in fish are important; fish from freshwater bodies receiving industrial effluents have been reported to be unfit for human consumption due to high tissue levels of some heavy metals [16-17], [8], [18-19]. To safeguard aquatic biota, trace element contamination levels must be determined using chemical biomonitoring and the study of biomarkers that constitute early indications of biological consequences [4]. Certain fish species may be better bioindicators of heavy metal contamination than others [20-21].

MATERIALS AND METHODS

Sequence retrieval: The target protein sequence of the growth hormone of *Oreochromis niloticus* (AAM50320.1) was retrieved from NCBI database in FASTA format and the respective mutated positions were changed manually.

Protein sequence function analysis: The protein sequence was analyzed using ScanProsite tool in order to identify the functional Domain and Motif regions.

Protein modelling and validation: The protein sequence of the selected growth hormone of *Oreochromis niloticus* was converted into 3D structure using an automated protein homology modelling server called Swiss Model server and the 3D structure was validated using ProCheck server for 3D structure quality assessment.

3D structure visualization: The modelled protein structure of the growth hormone of *Oreochromis niloticus* was viewed using an advanced molecular visualization software named Discovery Studio software.

Metal binding sites prediction: The predicted 3D structure was applied into MIB server to determine the potential non-essential metals () bound to the 3D structure of the growth hormone.

RESULTS AND DISCUSSION

>AAM50320.1 growth hormone [*Oreochromis niloticus*]
MNSVVLLLSVVCLGVSSQQITDSQRLFSIAVNRVTHLHLLAQRLFSDF
ESSLQTEEQRQLNKIFLQDFCN
SDYIISPIDKHETQRSSVLKLLSISYGLVESWEFPSRSLSGGSSLRNQISP
RLSELKTGILLLIRANQDE
AENYPDTDTLQHAPYGNYYQSLGGNESLRQTYELLACFKKDMHKVE
TYLTVAKCRLSPEANCTL

Fig 1 Protein sequence of *Oreochromis niloticus*

The above picture shows the FASTA format of *Oreochromis niloticus*

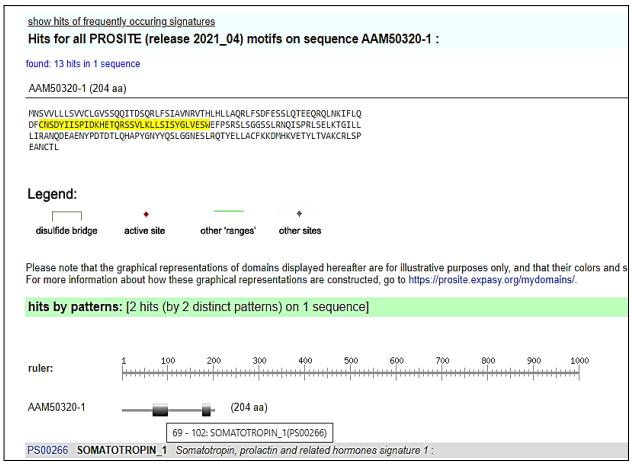


Fig 2 Protein sequence analysis of *Oreochromis niloticus*

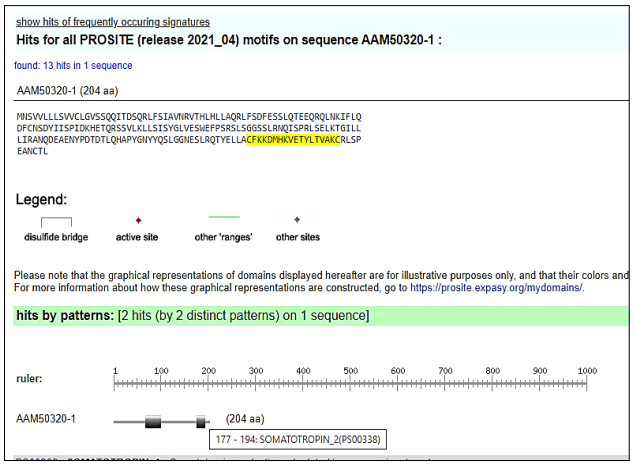


Fig 3 Protein sequence analysis of *Oreochromis niloticus*

The above picture [Fig 2] shows motif 1 present in the growth hormone of *Oreochromis niloticus* highlighted in yellow colour. The above picture [Fig 3] shows motif 2 present in the growth hormone of *Oreochromis niloticus* highlighted in yellow colour.

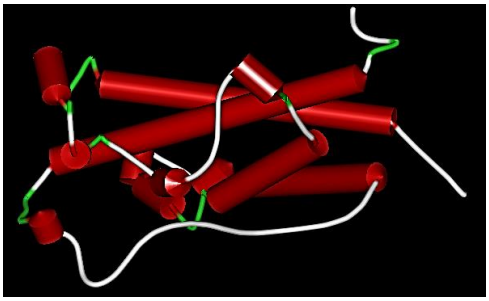


Fig 4 3D structure prediction of *Oreochromis niloticus*

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

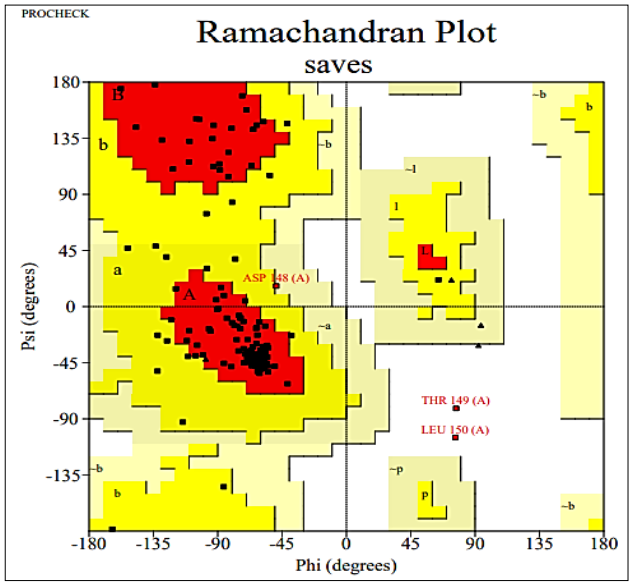
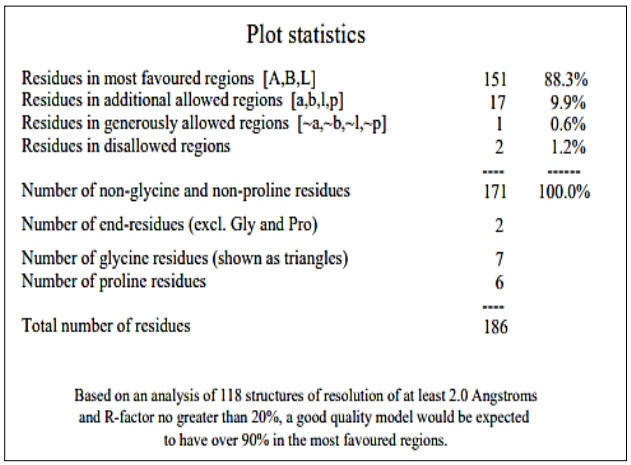


Fig 5 3D structure validation



Plot statistics

Residues in most favoured regions [A,B,L]	151	88.3%
Residues in additional allowed regions [a,b,l,p]	17	9.9%
Residues in generously allowed regions [~a,~b,~l,~p]	1	0.6%
Residues in disallowed regions	2	1.2%
Number of non-glycine and non-proline residues	171	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	7	
Number of proline residues	6	
Total number of residues	186	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.

The above picture gives details on the assessment of Ramachandran plot which shows the structural quality of the modelled protein.

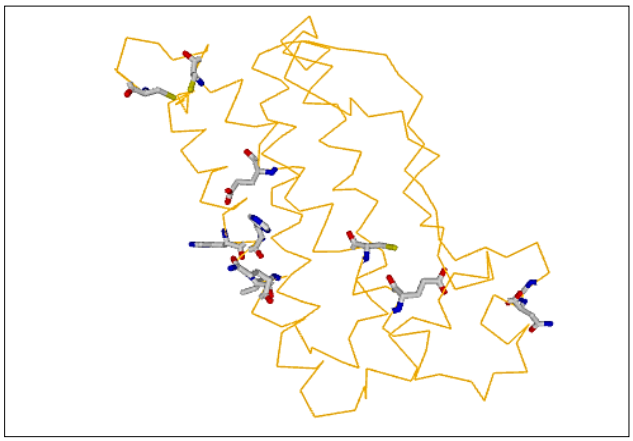


Fig 6 Heavy metal binding prediction

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

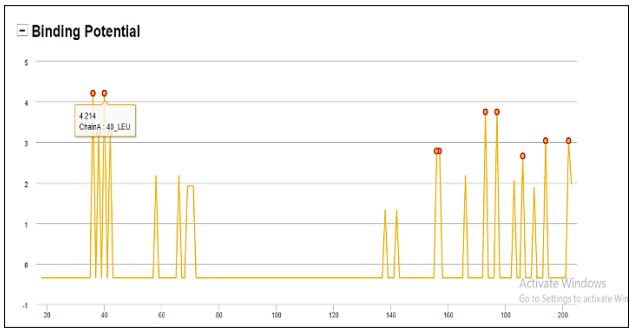


Fig 7 Heavy metal binding prediction

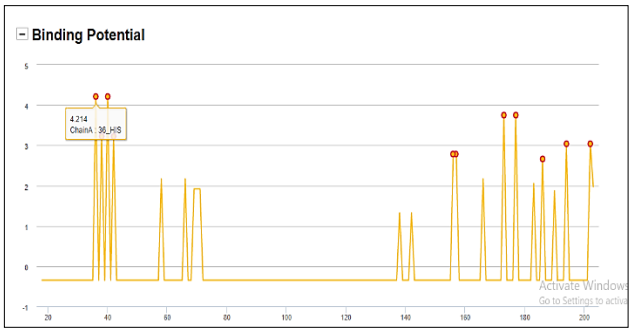


Fig 8 Heavy metal binding prediction

The above graphical picture [7] shows the binding potential of Leucine (40) of the growth hormone with the metal Hg and the another graphical picture [8] shows the binding potential of Histidine (36) of the growth hormone with the metal Hg.

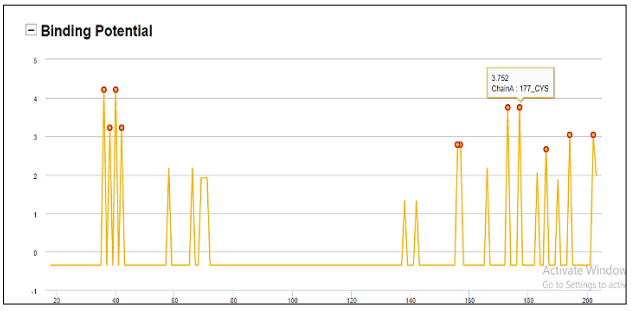


Fig 9 Heavy metal binding prediction

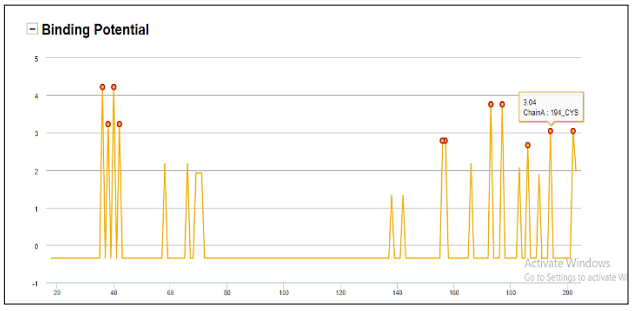


Fig 10 Heavy metal binding prediction

The above graphical picture [Fig 9] shows the binding potential of Cysteine (177) of the growth hormone with the metal Hg and another graphical picture [Fig 10] shows the binding potential of Cysteine (194) of the growth hormone with the metal Hg.

Fig 11 Heavy metal binding prediction

No.	Binding residues
1	36H, 40L
2	173E, 177C
3	38H, 42Q
4	194C, 202C
5	156G, 157N
6	38H, 186E

The above picture shows the various amino acid positions at which the metal is bound to the growth hormone's protein sequence. Here, we show that 36H and 40L are the best binding positions based on the graphical information (Fig 6).

The below picture [Fig 12] shows the schematic structure in secondary structure colour along with the respective heavy metal binding sites (Spacefill model represents Mercury (Hg) and yellow lines on the schematic model represents the positions Leucine 40 and Histidine 36) viewed using Discovery Studio Software.

The below picture [Fig 13] shows the schematic structure in secondary structure colour along with the respective heavy metal binding sites (Spacefill model represents Mercury (Hg) and yellow lines on the schematic model represents the positions Leucine 40 and Histidine 36) viewed using Discovery Studio Software.

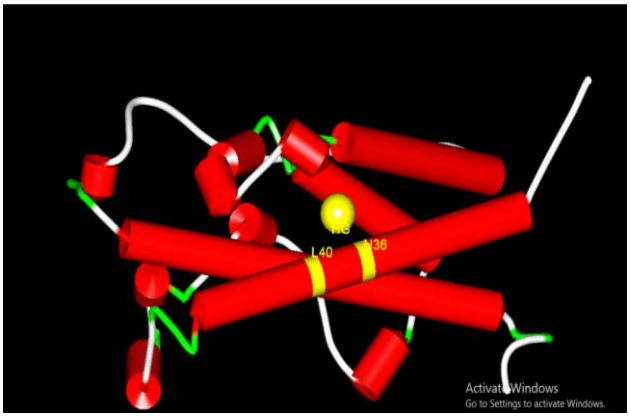


Fig 12 3D heavy metal binding prediction

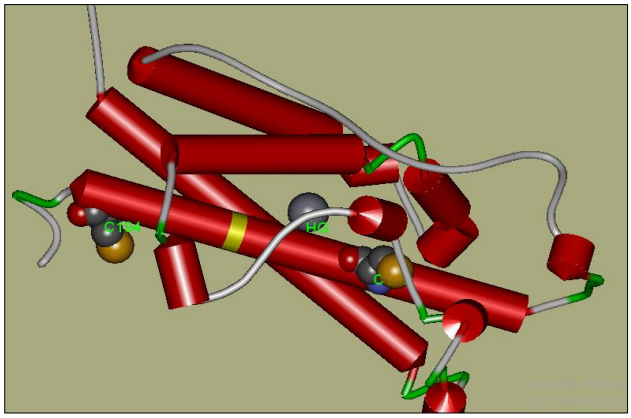


Fig 13 3D heavy metal binding prediction

Table 1 Metal binding summary

Target species (*Oreochromis niloticus*)	Heavy Metal	Motifs regions
36 H (Histidine) (Fig 6) 40 L (Leucine) (Fig 6) 194 (Cysteine) (Fig 7) 177 (Cysteine) (Fig 7)	Hg-Mercury	69 -102 (Motif 1) 177-194 (Motif 2)

The above table mentions that HIS (H) 36 and LEU (L) 40 are responsible for Mercury (Hg) heavy metal binding with the growth hormone of Tilapia.

The growth hormone of the tilapia fish, *Oreochromis niloticus* (CAA72415.1) (tigH gene), was downloaded in FASTA format from the NCBI GenPept database (Fig 1). For protein sequence analysis, we use SCANPROSITE tool in order to identify the motif regions present in the growth hormone of *Oreochromis niloticus*. (Fig 2-3, Table 1) shows motif 1 and motif 2 present in the amino acid sequence of the growth hormone (69 -102 (Motif 1), 177-194 (Motif 2)). The predicted motifs play a functional role in the growth hormone of *Oreochromis niloticus*.

The protein sequence of the growth hormone was modelled using Swiss Model Server. SWISS-MODEL was used in this investigation to translate the amino acid sequence of (*Oreochromis niloticus* –gH hormone protein) into a three-dimensional structure [Fig 4]. For docking, SWISS-MODEL [22-25] was utilized to thoroughly examine the molecular and structural features of the gH hormone protein. SWISS-MODEL is a server for automated three-dimensional (3D) protein structure comparative modelling. The SWISS-MODEL server homology modelling pipeline, which depends on ProMod3, an in-house comparison modelling engine based on Open Structure, was used by Waterhouse et al. to calculate models. The Ramachandran Plot of the simulated 3D protein was fully evaluated using the ProCheck server [26]. The 3D structure of the mutant protein was confirmed using the ProCheck server after modelling. (Fig 5) illustrates the Ramachandran Plot analysis, which demonstrates that the simulated protein is error-free (88.3%).

Mercury binds to the possible amino acids (36 H (Histidine) 40 L (Leucine) 194 (Cysteine) 177 (Cysteine)) (Table 1) of Oreochromis niloticus growth hormone, according to our Insilico research findings (Tilapia) (Fig 6-12). These amino acids are involved in metal absorption in the functional part of Tilapia fish, preventing the growth hormone from executing its regular duties.

We use MIB server [27] for the Prediction of metal binding in order to discover heavy metal binding amino acid sites. In the regular metabolism of fish, essential metals play a critical role. Their organs, on the other hand, may accumulate non-essential metals [27]. Essential metals include Fe, Cu, Zn, and manganese (Mn), while non-essential metals include Hg, Pb, nickel (Ni), and cadmium (Cd) [28]. Heath [29] found that increased heavy metal concentrations in fish's bodies have a negative impact on their growth and development during early life stages like hatching, larval development, and juvenile growth because they are more sensitive to these metals during these stages than they are during adult stages. Fish, obviously, create a pathway for dangerous heavy metals to get from the water to humans [30]. When ingested in excess of the allowed dose, trace elements can be toxic (acute, chronic, or sub-chronic), and heavy metals can be neurotoxic, carcinogenic, mutagenic, or teratogenic. Convulsions, vomiting, ataxia, paralysis, gastrointestinal problem, diarrhoea, hemoglobinuria, tremor, stomatitis, pneumonia, and depression are common symptoms of metal [e.g., Cd, Pb, As, Hg, Zn, Cu, and aluminium (Al)] poisoning in humans [31].

CONCLUSION

In this in silico study, we have identified the potential amino acids involved in binding with non-essential metals like Mercury (Hg) which inhibit the growth of Tilapia fish (*Oreochromis niloticus*) in rivers. Our results clearly elucidate that the functional part (motifs) in the protein sequence of the growth hormone act as metal binding regions. We conclude, that our study would play a vital role in fish farming industries to take preventive measures against water being polluted by non-essential metals to enhance the growth of fish.

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