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Original article

Possibility of myoglobin as a molecular marker for phylogenetic relationship of fish

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Introduction

Fish show a large biodiversity in the strategies to adapt to respective inhabiting environments. In order to know the genetic relationship among fish species, many attempts have been made to identify and classify the genetic relationship based on some molecular markers such as mitochondrial cytochrome *b* (cyt *b*) and cytochrome *c* oxidase subunit I (COI) genes. Not all of them are, however, successful for this purpose. In the previous study, we found the availability of muscle tropomyosin (TM) for this purpose [1]. In this study, the possibility of myoglobin as a new molecular marker for the above purpose classification and phylogenetic analysis of fish was evaluated.

Materials and methods

Amino acid sequence
The sequence data of fish muscle Mb, cyt *b*, and COI were collected from the database in National Center for Biotechnical Information (NCBI); medaka (*Oryzias latipes*), bluefin tuna (*Thunnus thynnus*), zebrafish (*Danio rerio*), Atlantic salmon (*Salmo salar*) and tilapia (*Oreochromis mossambicus*), whereas the sequence of mouse (*Mus musculus*) was used as an outgroup. The species and accession numbers of amino acid sequences are shown in Table 1.

Table. 1. The species and amino acid sequences from NCBI

Species	Mb	Cyt *b*	COI
Tuna (*T. thynnus*)	AAG02105	ABS85054	BAC78532
Tilapia (*O. mossambicus*)	BAH22119	ABB85081	AAT92269
Medaka (*O. latipes*)	XP_004065750	BAV60900	BAH84895
Zebrafish (*D. rerio*)	AAH56727	ALK26838	NP_059333
Salmon (*S. salar*)	ACM09229	ACB30582	AAF61380
Mouse (*M. musculus*)	NP_001157520	YP_001686710	NP_904330

Phylogenetic analyses
The amino acid sequences of TM, cyt *b*, and COI were subjected to phylogenetic tree construction including those of some other representative fish species. The phylogram constructed by the maximum likelihood, neighbor-joining and Unweighted Pair Group Method with Arithmetic Mean (UPGMA). The three methods were based on CLUSTAL W which generated paired alignments of all sequences. Bootstrap majority consensus values on 1000 replicates were calculated with CONSENSE and are indicated in percent at each branch node. All these programs are parts of Molecular Evolutionary Genetics Analysis Ver. 7 (MEGA 7). The evolutionary distances were computed using the JTT matrix-based method.

Results

The phylogenetic trees drawn based on Mb sequences were similar to those by the traditional classification based on the other markers. The primary and secondary structures of Mbs were similar to each other, but were clearly distinguishable among the five species. Such differences in structures would be greatly involved for adaptation of Mb molecule to the physiological conditions of each species. From these results, it is suggested that Mb can be a molecular marker for the phylogenesis of fish, but was found to be slightly inferior to TM.

Discussion

If the data from a larger number fish species could be included, the results might have been much closer to the actual relationship. However, the databank of fish cannot provide so much data at present. Based on the results, the Mbs could be a one of good biomarkers for fish evolution studies. The phylogenetic trees drawn based on Mb sequences seemed better compared to those by cyt *b* or COI.

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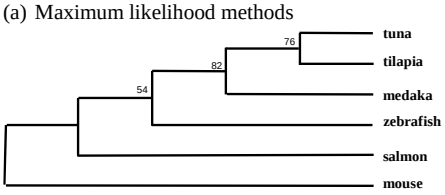
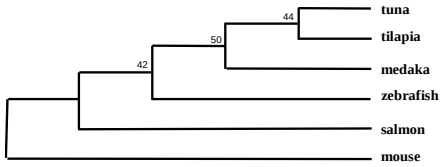
(a) Maximum likelihood methods



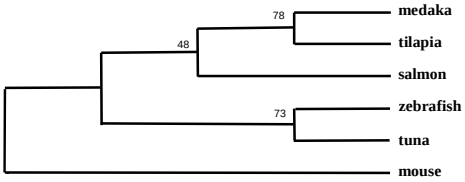
(b) Neighbor-joining methods



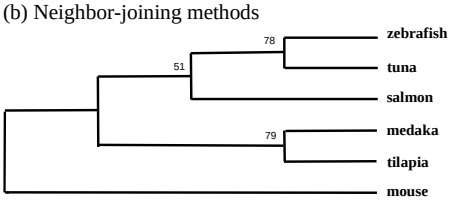
(c) UPGMA methods

Fig. 4. Phylogenetic trees based on the amino acid sequences of myoglobins from various fish species. Deduced amino acid sequences were aligned using CLUSTAL W, and the trees were constructed by the maximum likelihood (A), neighbor-joining (B), and UPGMA methods (C). Mouse was used as the outgroup. The percentage of replicated tree in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The evolutionary distances were computed using the JTT matrix-based method. Evolutionary analyses were conducted in MEGA 7.

(a) Maximum likelihood methods



(b) Neighbor-joining methods



(c)UPGMA methods

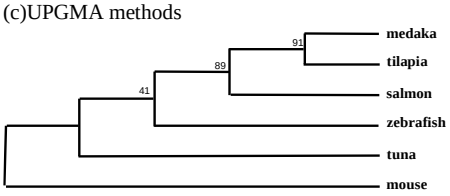
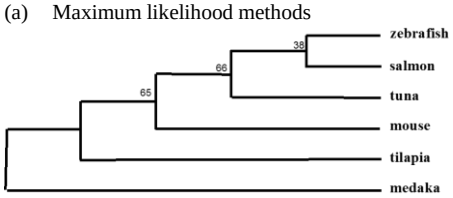
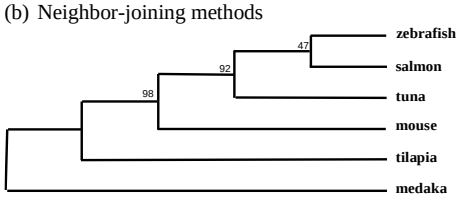


Fig. 5. Phylogenetic trees based on the amino acid sequences of cyt *b*s from various fish species. Deduced amino acid sequences were aligned using CLUSTAL W, and the trees were constructed by the maximum likelihood (A), neighbor-joining (B), and UPGMA methods (C). Mouse was used as the outgroup. The percentage of replicated tree in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The evolutionary distances were computed using the JTT matrix-based method. Evolutionary analyses were conducted with MEGA7.

(a) Maximum likelihood methods



(b) Neighbor-joining methods



(c) UPGMA methods

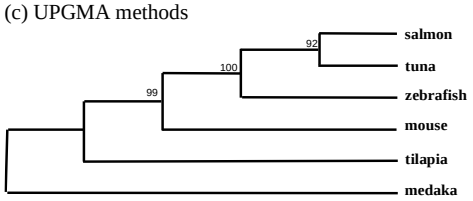


Fig. 6. Phylogenetic trees based on the amino acid sequences of COIs from various fish species. Deduced amino acid sequences were aligned using CLUSTAL W, and the trees were constructed by the maximum likelihood (A), neighbor-joining (B), and UPGM methods (C). Mouse was used as the outgroup. The percentage of replicated tree in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The evolutionary distances were computed using the JTT matrix-based method. Evolutionary analyses were conducted in MEGA7.

Conclusions

Based on the results, Mb could be a one of good biomarkers for fish evolution studies. The phylogenetic trees drawn based on Mb sequences seemed better compared to those by cyt *b* or COI.

Acknowledgements

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References

1. You YC, Huang MC (2016) Int J Sci Engin 6: 23–42