Analysis of fragment homology among 331bp myoglobin of green turtle (Chelonia mydas) with hypoxia-tolerant and hypoxia-intolerant organisms

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Abstract. Myoglobin protein plays significant role in intracellular oxygen storage and transcellular oxygen transport in the green turtle (Chelonia mydas). This globular protein contains amino acid compositions which have similarities between other species. This study aimed to analyse the fragment homology among 331bp myoglobin sequences of green turtle with hypoxia-tolerant and hypoxia-intolerant group organisms. Green turtle myoglobin sequences was aligned with 16 organisms for 84 amino acids number 71 to 154 in the N terminal region of the myoglobin protein. The data was analysed in BLASTX bioinformatics portal. The result showed that the percentage of similarity was 58%-93% with myoglobin in hypoxia-tolerant organisms and 79%-93% in hypoxia-intolerant organisms. Fragment homology in this study indicate the conserved positions of protein sequences during evolution in hypoxic tolerant organisms, which indicate that these positions may be important for the structure or function of myoglobin. Further study is needed to determine the difference of amino acid composition between these organisms.

Keywords: Genetic diversity, hypoxia, oxygen homeostasis, sea turtle conservation

1. Introduction
Myoglobin is a hemoprotein that is commonly found in skeletal and heart muscle cells, structurally composed of a single polypeptide chain of 154 amino acids [1]. Myoglobin has a comparative structure and function with hemoglobin, which can bind oxygen reversibly so that it may be able to facilitate the transport of oxygen from red blood cells to the mitochondria. This role is more commonly seen in marine mammals and marine reptiles that have greater chances of experiencing hypoxia. One of them is a green turtle (Chelonia mydas) [2, 3].

Green turtle classified as animals that are tolerant to hypoxia [4]. In its life phase, green turtle hatchlings are exposed to a hypoxic environment when they hatch deep down in the sand until they reach the surface [5]. Exposure to the hypoxic environment also occurs when green turtles dive. To deal with low oxygen environment, hypoxia-tolerant animals carry out adaptation mechanisms by increasing levels of myoglobin in muscle tissue [6]. The high concentration of myoglobin during diving helps to store oxygen in skeletal muscle cells which are significant during the process of aerobic metabolism [3].
In addition to its role in intracellular oxygen transport, myoglobin can be used as a marker to indicate the level of homology between one species and another. This is because myoglobin is a homologous protein that has a similar amino acids series between species [7]. Animals containing these homologous proteins may have the same evolutionary origin. The closer level of homology shows the fewer structural differences in protein.

Differences between homologous proteins can be detected in several ways, for example based on immunological reactions. This analysis technique is related to antibody specificity to the antigen. Antibodies are specific to antigens that stimulate their formation and antigens of species that have homologous proteins with them. Through immunological reactions, it can be directly known the structural characteristics of a protein that is being analysed. One technique can be applied for the purpose of homology studies. Immunological techniques that can be performed to analyze the structural characteristics of proteins, such as immunoblot techniques, ELISA (Enzyme Linked Immunosorbent Assay), and Western blots [8]. This study aimed to analyse the fragment homology among 331bp myoglobin sequences of green turtle with hypoxia-tolerant and hypoxia-intolerant group organisms. The study of homology among these organisms is important to determine the genetic relationship between them and their ability to adapt in hypoxia environment.

2. Materials and methods

As shown in figure 1, the sequence of 331bp myoglobin cDNA which collected from green turtle (C. mydas) under ethical clearance number LB.03.02/KE/1479/2008 from Ministry of Health, Indonesia also from CITES Certificate number 315631/02/2008 released by Bristol UK and certificate number SK/136/IV-SET/2008 from Ministry of Environment and Forestry’s Directorate General of Forest Protection and Nature Conservation, Indonesia [9]. The 331bp cDNA fragment is a fragment of green turtle’s cDNA which expressed the myoglobin protein in several conditions, such as in low oxygen environment. The myoglobin data of animals from hypoxia-tolerant group and hypoxia-intolerant group were randomly selected from NCBI gene bank. The sequence alignment was conducted on the BLASTX program from the NCBI site. The fragment of the existing myoglobin amino acids from the green turtle that will be analyzed was inserted into the first test box, then in the second test box were inserted the data of myoglobin amino acid from test organism one by one. The data were entered into the table and analyzed. The similarity percentage among green turtle myoglobin fragment and other organisms was analyzed by descriptive statistics to determine the highest and lowest percentage among them.

Figure 1. The 331bp myoglobin fragment of green turtle (C. mydas)

3. Results and discussion

Alignment analysis between the green turtle’s myoglobin fragment and 16 test organisms for 84 amino acids number 71 to 154 in the N terminal region of the myoglobin protein was carried out by bioinformatics computerisation. Test organisms were divided into two groups, hypoxia-tolerant group organisms and hypoxia-intolerant group organisms. Hypoxia-tolerant groups in terms of an organism’s ability to survive and their ability to adapt in low oxygen conditions [10–12]. Hypoxia-tolerant organisms, for example, are whales, turtles, liver worms, zebras, carp, tuna, and penguins (table 1). While the criteria for hypoxia-intolerant organisms are organisms that cannot live longer and unable to
adapt in a low oxygen environment, for example are humans, orang utans, bulls, pigs, and iguanas (table 2).

**Table 1.** The result of alignment analysis between the 331bp myoglobin of green turtle (*C. mydas*) with hypoxia-tolerant organisms.

| No | Accession Number | Group       | Species              | Similarity (%) |
|----|------------------|-------------|----------------------|----------------|
| 1  | AAB29314.1       | Reptiles    | *Caretta caretta*    | 93             |
| 2  | NP_956880        | Fish        | *Danio rerio*        | 58             |
| 3  | P02199           | Birds       | *Aptenodytes forsteri* | 91         |
| 4  | BAF03579         | Mammals     | *Physeter catodon*   | 83             |
| 5  | P02204           | Fish        | *Cyprinus carpio*    | 62             |
| 6  | AAG02112.1       | Fish        | *Thunnus albacares*  | 82             |

The homology test results showed that 84 amino acid building units in the region before N terminal myoglobin of the green turtle had a homological range at 58%-93% with myoglobin fragment of hypoxia-tolerant organism. Also, the homology results showed a homology range at 79%-93% with myoglobin of non-hypoxic tolerant organisms. The mean of myoglobin homology similarity of the green turtle with the fish group, reptiles, birds, mammals, and invertebrates in a row is: 67.3%, 89.5%, 88.3%, 82%, and 0%. The 331bp green turtle myoglobin fragment is most similar to *Caretta caretta* and less similar to the invertebrates. It can be seen that the myoglobin fragment similarity interpreted the phylogenetic relationship among green turtle and other organisms.

The results showed that there were differences in amino acid residues of 16-21% at 84 amino acids on the side of N myoglobin with groups of test organisms (table 1 and table 2). Unfortunately, these different amino acid sequences cannot be analysed because their locations are randomly distributed along the terminal N of myoglobin protein. However, it was suspected that the difference in amino acid resistance is outside the important residual region of oxygen binding to the myoglobin molecule.

**Table 2.** The result of alignment analysis between the 331bp myoglobin of green turtle (*C. mydas*) with hypoxia-intolerant organisms.

| No | Accession Number | Group        | Species               | Similarity (%) |
|----|------------------|--------------|-----------------------|----------------|
| 1  | NP_999401.1      | Mammals      | *Sus scrofa*          | 80             |
| 2  | AAH18001.1       | Mammals      | *Homo sapiens*        | 79             |
| 3  | 100054434        | Mammals      | *Equus caballus*      | 80             |
| 4  | NP_001125556.1   | Mammals      | *Pongo abelii*        | 83             |
| 5  | NP_001157520     | Mammals      | *Mus musculus*        | 84             |
| 6  | NP_067599        | Mammals      | *Rattus norvegicus*   | 84             |
| 7  | NP_776306        | Mammals      | *Bos taurus*          | 83             |
| 8  | NP_001161224     | Birds        | *Gallus gallus*       | 92             |
| 9  | JC7789           | Birds        | *Accipiter gentilis fujyamae* | 82 |
| 10 | ABN71515.1       | Reptiles     | *Iguana iguana*       | 86             |
| 11 | CAA55885         | Invertebrates| *Aplysia limacina*    | 0              |
| 12 | AMM18464         | Invertebrates| *Clonorchis sinensis* | 0              |

In bioinformatics, testing the homology of green turtle’s myoglobin at 84 N-side amino acid residues with the test mammal group gave 84% homologies with mouse and 79% with humans. This means that the molecular structure of myoglobin on the N side of myoglobin has much in common with rat myoglobin [13] and humans [14].

Rainbow trout (*Oncorhynchus mykiss*) from hypoxia-intolerant group and carp (*Cyprinus caprio*) which is hypoxia-tolerant animal share common similarities in the location of myoglobin on cellular level [15]. Gene expression in the carp’s non-muscular tissue related to the protein expression in the
liver. Exposure to hypoxia on this animal causes increasing the level of myoglobin transcription in non-muscular tissue. The same thing happens to the green turtle in the hatching phase. Exposure to the hypoxia environment at that time led to the need for myoglobin expression on some non-muscular tissues [11,16]. Whereas in hypoxia-intolerant animal, such as rats and rainbow trout, the abundant level of myoglobin mostly found on cardiac and skeletal tissues [9,15].

A homologous sequence does not necessarily exhibit significant similarity. In the analysis of this data, there were thousand possibilities of homologous protein sequences that were not significant, but it can be stated as homolog because the structural similarities shown through statistic data were significant enough or there were strong sequences similarity [17]. The recent study showed that the homology between two sequences signify the similarity of structure and often indicate the similarity of function, but the relation between homology and similarity of function are less reliable because the single structure of protein can be expressed for various functions [17, 18].

4. Conclusion
The fragment homology between test organisms and the green turtle indicate the position of protein sequence that is derived and preserved during the evolution of this group. Further research is needed to determine the differences in composition of amino acids between these organisms and compare the differences of structure and function between the myoglobin protein of these organisms.

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