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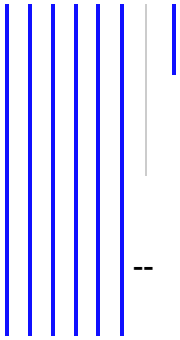

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ANALYSIS OF MYOGLOBIN DNA FRAGMENT HOMOLOGY ALONG 114BP IN SOME KIND OF MARINE ANIMAL WHICH ABLE TO LIVE IN OXYGEN MINIMUM ZONE

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ABSTRACT

Myoglobin is a hemoprotein that contributes to intracellular oxygen storage and facilitates oxygen diffusion into the mitochondria. Myoglobin production will increase along with low levels of environmental oxygen called hypoxia. Myoglobin is also a homologous protein, which can be used to show the relationship level between the species. This research aims to identify and determine the relationship status between green turtle, mackerel tuna, butterfly stingray, and hammerhead shark based on the molecular of myoglobin DNA fragment nucleotides composition along 114bp. This descriptive study was conducted using PCR and electrophoresis as the methods. These results indicated that nucleotide sequences of DNA fragments myoglobin along 114bp on green turtles, mackerel tuna, stingrays butterfly and hammerhead sharks have been identified. The percentage of nucleotide homology at the myoglobin DNA fragments along 114bp on green turtle are 92% (mackerel tuna), 88% (butterfly stingray) and 75% (hammerhead shark) while the percentage of amino acid sequence homology of myoglobin DNA fragments along 114bp on green turtle at 79% (mackerel tuna), 76% (butterfly stingray), and 47% (hammerhead shark). Cladogram are formed based on the nucleotide and amino acid sequence showed the green turtle has a closest relation with mackerel tuna while the butterfly stingray has the closest relation with hammerhead shark.

KEYWORDS: Myoglobin, Homology, DNA, Amino Acid , Genetic Relationship.

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I. INTRODUCTION

Myoglobin is hemoprotein which has iron (Fe) from a heme group[1]. Myoglobin plays a role as O_2 storage and facilitates O_2 diffusion. Myoglobin will bind oxygen in high affinity when the amount of oxygen in blood is enough to transfer electrons in mitochondria, when partial pressure of oxygen is low, myoglobin will release oxygen and provide oxygen diffusion from sarcolemma to mitochondria. The concentration of myoglobin will increase when the oxygen levels is low [2]. Myoglobin is a protein used to indicate relation levels between the species due to its amino acid sequence homolog (homolog sequence)[3].

The oceans has low oxygen levels zone. The zone is known as Minimum Oxygen Zone lay at depth 500-2500 meters above sea level [4]. Low oxygen levels in oceansis caused by poorly oxygen circulation in the zone. In the region of tropical North Atlantic Ocean, the minimum oxygen zone is at depth of 200-700 meters above sea level [5].

In the oceans, Pelagic zone belongs to Minimum Oxygen Zone. It is known by the decline of pelagic fish habitats as the result of minimum oxygen zone [6]. Marine animals which capable to live in minimum oxygen zone are Green Turtle, Mackerel Tuna, and Hammerhead Sharks. These marine animals are capable to live at depths of more than 200m below the sea levels. Some of marine animals which has a high myoglobin level are Mackerel Tuna, Tuna, and Sardine [7]. Mygolobin is essential protein for animals to live in low oxygen levels environment (hypoxia).

There are no information discussed about myoglobin charaterization of animals live in minimum oxygen zone. The initial step to understand myoglobin characterization is by detecting nucleotide base sequence of DNA fragments and analyzing nucleotide homolog and amino acid of myoglobin DNA fragments. The Molecular mark of base nucleotide sequence from myoglobin DNA fragments in tutrle green has known to be 114pb and decoded into 38 amino acids in 63-100 of N Terminal area [8]. N terminal of myoglobin can be used as mark molecular due to its amino acids residue is a mark moleculer of myoglobin protein of vertebrata.

One of the program used to analyze nucleotide homolog as well as protein in organism is bioinfomatic BLAST (*Basic Local Aligment Search Tool)*. BLAST is a program used to compare nucleotide sequences and protein with the database in order to calculate the comparison of statistical significancy, either used to conclude the relating evolution between DNA sequence and peptides sequence.

Based on the molecular mark, base nucleotide sequence will be detected in the same myoglobin genes fragments from green turtle, mackerel tuna, stingray butterfly and hummerhead shark [9]. All nucloetide sequence of myoglobin DNA fragments along 114pb in green turtle, mackerel fish, stingray butterfly and hummerhead shark can be detected from the result of mark molecular detection.

II. MATERIALS AND METHODS

This is a descriptive research. Data of Nucleotide sequence along 114pb of myoglobin DNA fragments was analyzed by bioinformatic program such as BLAST N, BLAST X, and Clutal W2. The sample used are 40mg muscle tissues from each green turtle, mackerel tuna, stingray butterfly and hummerhead shark.

2.1 Isolation of Genom DNA

DNA isolation was done using Wizard Genomic Purification Kit. The muscle samples were destroyed using mortar. The 40mg of destroyed muscle samples then put into a micro tube. Futhermore, isolation of Genom DNA was done by Wizard Genomic Purification Kit protocol with some modification i.e. increase K proteinase and setincubation temperature at 55^0C. Purity index and concentration of isolated samples were determined using Nanodrop Maestro Spectrophotometer. High concentration and pure genom DNA was stored in coolant-case at -4^0C to be used later on PCR process.

2.2 Amplification of DNA fragments of Myoglobin along 114pb

Amplification of DNA fragments of myoglobin along 114pb was done by kit Taq Polimerase: KAPA Taq Ready Mix. Primer used to obtain DNA fragments of myoglobin along 114pb are : Primer reverse (Myo Turtle R1) : (5’ gat ttt atg ctt ggt ggc atg gc 3’), and Primer

Forward (Myo Turtle F2) : (5' aag aag cat gga act act gtc 3'). DNA concentration template 100ng/ul and annealing temperature at 58^0 C, with 40 times cycle.

2.3 Myoglobin DNA fragments along 114pb Detection

DNA fragments of myoglobin along 114pb was detected using agarose 2% electrophoresis methode. The result of PCR as many 50µL and 5µL marker DNA (*KAPA Universal Ladder*)was added into agarose well. Electrophoresis was operated with 100V during 55 minutes. Visualization was done by UV-transilluminatir. The result which formed positive band along 114pb was documented then positive band inside the agarose was cut and put into microtubes.

2.4 Gel purification by *QIAquick Gel Extraction* Kit.

Agarose gel that contains positive band as many 300mg was purified using *QIAquick Gel Extraction* Kit. Purification process was done according to *QIAquick Gel Extraction* Kit protocol. The Concentration and the purity of purified DNA was determined using Nanodrop Maestro spectrophotometry, then samples with good purity (Concentration DNA comes to 50ng/µL and purity > 1,8) were sequenced.

2.5 Myoglobin DNA fragments homolog analysis

Sequencing yields in the form of myoglobin DNA fragments along 114pb were analyzed using bioinformatic program i.e. BLASTXN (nucleotide) and BLASTX (amino acids). Whereas, cladogram used to determine the relation between species as hypoxia tolerant animals according to myoglobin DNA fragments made using Clustal W2 software.

III. RESULT

3.1 Genom DNA

The isolation yield has purity index more than 1,8 and high concentration (>100ng/µL). The highest concentration of DNA is 213,48ng/µL in stingray butterfly sample**,** while the lowest concentration of DNA is 168,55 ng/µL in hammerhead shark sample (Table 1).

3.2 Visualization of PCR Result

DNA fragments of myoglobin along 114pb was successfully amplified at 58^0C followed by 40 times cyclus and showed thick density of positive band. The visualization from PCR result in agarose 2% can be seen in Fig 1 and 2.

3.3 The homology of nucleotide and amino acid of myoglobin DNA fragment along 114pb

The Homology test yields showed that nuclotide sequence of the samples has range from 75-99% compared to nucleotide sequence of green turtle myoglobin in NCBI. While homolog range of amino acid is 47-100% compared to nucloetide sequence of green turtle in NCBI (Table 2).

The alignment of nucleotide between the samples showed that 81 nucleotides from myoglobin DNA fragments and 17 amino acid residues in four samples have no significance difference . The alignment nucleotide sequence between samples is showed in Fig 3, while the amino acid alignment in Fig 4.

IV. DISCUSSION

Collecting supernatan from DNA isolation process using homogenat-making technique has an effect to the yields. Based on that matter, the isolation process was done by destroying the tissues using a mortar. Mortar is a traditional tool used to destroy tissues as well as homogenat-making[10]. The adventage of mortar in destroying tissues is that the yield become softer particles but the quantity of the tissues reduced. Therefore, the quantity of sample has to be more in the process of destroying sample. Other than changing the way of destroying a sample, there are some modification such as increasing the quantity of the sample twice than before, increasing K proteinase and setting the incubation temperature at 55^0C.

Increasing of K proteinase aims to degrade a protein. K proteinase is a proteinase enzyme which is extracted from *Tritirachium album*. Proteinase is an enzyme that can change a protein into peptides and be a protecting agent in DNA extraction process by activating endrogen nuclease [11]. Based on measuring purity index and concentration using Maestro Nanodrop tool showed that DNA genom obtained has good purity index (>1,8). A good purity index of DNA

has range from 1.8-2.0 [12]. Generally, The result of DNA isolation contains RNA, Protein as well as polysaccharides. RNA Contamination can be eliminated using RNA degrading enzyme or RNAse[13].

The success of CPR technique is affected by annealing temperature. The attachment process of primer to DNA is effected by temperature accuracy in annealing process[14]. Primer used in CPR optimazation has Tm 60^0C for Myo Turtle F1 primer whereas Myo Turtle R1 has Tm 58^0C. Based on that matter, the range of annealing temperature is 54-64^0C. Annealing temperature used as primer has range between 3-5^0C from the average Temperature Melting (Tm)[15]. The optimal annealing temperature in resulting DNA fragments of myoglobin gen along 114pb is 58^0 C (Figure 1 and 2). Modification of CPR program can be done by modifying annealing temperature and increasing the cyclus in CPR reaction[16].

Based on alignment yields of nucleotide sequence and amino acid of myoglobin DNA fragment along 114pb in four samples showed a high percentage of homology due to myoglobin is part of homolog protein. Myoglobin is a homolog protein. Myoglobin protein has a constant and inconstant amino acid residu. Constant amino acid residue is a residue of acid amino in which all the species has it, while inconstant amino acid residue is a variation of amino acid sequence between species[3].

Myoglobin of mackerel tuna and hummerhead shark along with shark has low stability and easily denaturated due to their hydorphobicity [17]. The hidrophobicity is easily found in polar amino acid group. Polarity and the amount of acid amino in a molecul will determine hydrophobic level of protein molecule. The more hydrophobic of amino acid, the more unstable molecule is. In mackerel tuna and hummerhead shark, stability of myoglobin either caused by those animals only have 4 electrostatic bonds and 2 hydrogen bonds. While mammals and birds myoglobin have 8-9 salt bridges, 4 hydrogen bonds, and 11 electrostatic bonds [17].

The composition of hydrophobic amino acid residue in human and green turtle myoglobin are 42%. While mackerel tuna is 46% and hummerhead shark is 55%. It means,

myoglobin molecul of human and green turtle relatively stable compared mackerel tuna and hummerhead shark molecule.

Protein stability is affected by the composition of hydrophobic and hydrophilic residue. Protein stability is describe in statistic potential [18]. Statistic potential is affected by protein size and the composition of hydrophilic and hydrophobic amino acid residue. The increase of hydrophobic amino acid residue will enhance SVR. SVR increase will enlarge the protein size, reduce the stability and polar interaction in protein. Polar interaction between hydrophylic amino acid residue enhances protein stability by forming hydrogen bonds between aromatic amino acids as well as salt bridges. Beside, there are some obstacles in α helix formation i.e. the rigidity and transfiguration in trans peptide bonds, electrostatic forces between amino acid residue and R group charged, the size of R group, and the presence of proline [3].

The result showed that there were obstacles in polipeptides chain conformation due to some amino acid residue. In amino acid residue in green turtle, there are K-K-H (63-65), H-K (89-99), T-T (67-68), L-T (71-72), dan N–N (81-82) (Figure 4)that prevent α helix formation. K and H residues are a positive charged molecules of amino acids, the repulsion between the two molecules effect hydrogen bond stability[3]. The position between two N residues close to one another tends to prevent α helix formation due to N residue large size [3]. Same cases occur to amino acid recidue of myoglobin DNA fragments along 114pb from mackerel tuna, stingray butterfly and hummerhead shark .

Beside the same molecule charged and size, proline residue either prevent α helix formation [3]. The presence of proline residue increases the rigidity of polipeptides chains and prevents the α helix rotation [19]. Amino acids of myoglobin DNA fragments from green turtle, stingray butterfly and hummerhead shark have proline residue in 84 and 89 sites (Figure 4). Nitrogen atom in proline is a part of rigid chain, and the ring bond between N-C will never rotated [3].

Human myoglobin has genetic relationship woth anole lizard (reptile) myoglobin then followed by zebrafish (teloist fish)[20]. While *Zebra fish* myoglobin has fairly genetic relationship with houndshark (*Cartilagous fish*). Amino acid in amnoytes animals like human and reptiles has a high conserve value,

followed by teloist fish[21]. Those are reasons over the high percentage of homology between green turtle and mackerel tuna. Whereas, the reason of high homology percentage in the genetic relationship between stingray butterfly and hummerhead shark is because they're calilagous fish and in the same classes, Elasmobrancii.

V. CONCLUSION

Nucleotide sequence of myoglobin DNA fragments along 114pb was identified in green turtle, mackerel tuna, stingray butterfly and hummerhead shark. Myoglobin DNA fragments along 114pb in green turtle has high percentage of nucleotide homology toward myoglobin DNA fragments along 114pb in mackerel tuna (92%), stingray butterfly (88%), and hummerhead shark (75%). Myoglobin DNA fragments along 114pb in green turtle has high percentage of amino acid homology toward myoglobin DNA fragments along 114pb in mackerel tuna (92%), stingray butterfly (88%), and hummerhead shark (75%). Based on nucleotide homology percentage and amino acid of myoglobin DNA fragments along 114pb, green turtle has the closest genetic relationship with mackerel tuna, while stingray bbutterfly has genetic relatiobship with hummerhead shark.

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Table 1 The absorbancy yield of Nanodrop Maestro spectrophotometry with the concentration of Genom DNA

Sampel	A230 (nm)	A260 (nm)	A280 (nm)	A260/ A230 (nm)	A260/ A280 (nm)	[DNA] (ng/µl)
A	2,644	4,111	2,155	1,555	1,908	205,56
B	1,729	3,513	3,354	1.047	2,032	175.64
C	4,524	4,270	1,914	0,944	2,231	213,48
D	3,683	3,371	1,790	0,915	1,884	168,55

Sample:
A :green turtle
B: mackerel tuna
C: stingray butterfly
D. hummerhead shark

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Figure 1 positive band of green turtle sample in some annealing temperatures. (column M: *marker* DNA (Promega Ladder 100bp), column 1: 55°C, kolom 2: 56°C, column 3: 57°C, column 4: 58°C, column 5: 59°C, column 6: 60°C, column 7: 61°C, column 8: 62°C).

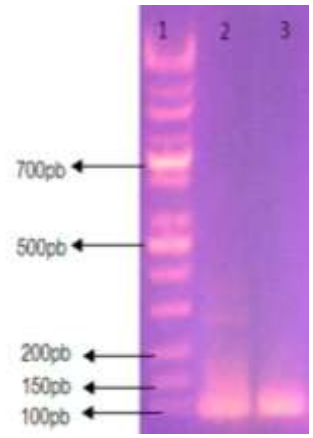

Figure 2 positive band in some samples (column 1: *marker* DNA (*Kappa Universal Leader 100bp*), column 2: hummerhead shark, kolom 3: mackerel tuna).

Tabel 2. The comparison between nucloitide homolog adn amino acid residue of myoglobin DNA fragments along 114pb.

Homology	Persentage Homology	
	Nucleotide	Amino Acid
Green Turtle- Green Turtle NCBI	99%	100%
Green Turtle NCBI- mackerel tuna	92%	79%
Green Turtle NCBI- stingray butterfly	88%	76%
Green Turtle NCBI- hummerhead shark	75%	47%
Mackerel tuna- stingray butterfly	80%	65%
Mackerel tuna- hummerhead shark	74%	44%

Stingray butterfly- hummerhead shark	73%	71%

Figure 3. The alignment from Nucleotide sequence of myoglobin DNA fragment along 114pb between four samples (Green turtle, mackerel tuna, stingray butterfly, hummerhead shark).

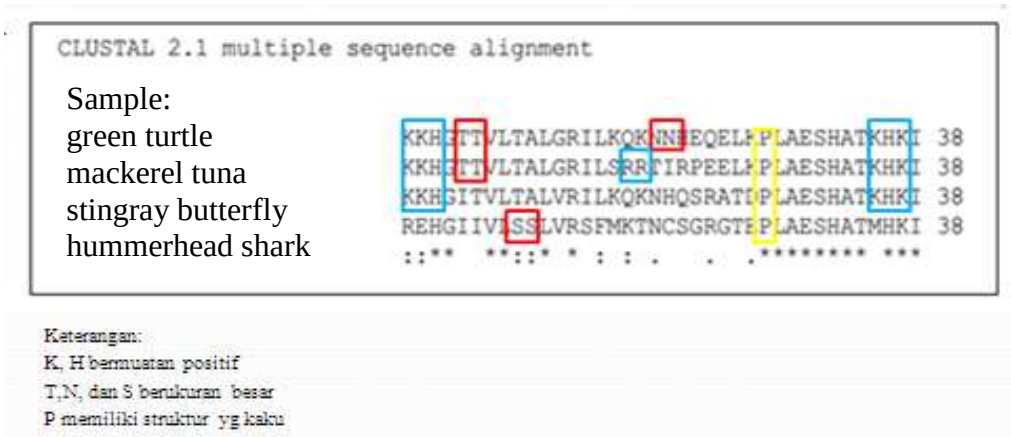

Figure 4. The alignment from amino acid sequence of myoglobin DNA fragment along 114pb between four samples (Green turtle, mackerel tuna, stingray butterfly, hummerhead shark).

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