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Hemoglobin Sequences of the Painted Turtle

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Introduction

Hemoglobin is the blood protein that carries O_2 from the lungs to the tissues, and returns CO_2 from the tissues to the lungs. Made of four subunits, each subunit is bound to iron-containing heme group. Its final quaternary structure determines its ability to bind O_2 under different O_2 and metabolic conditions. Upon binding O_2 , hemoglobin generally changes from a tense state to a more relaxed conformation; however, in turtles it remains in a strained conformation for all physiological conditions (Reid 2007).

The hemoglobin of adult turtles is compromised of two α-globin (α-D and α-A) and two β-globin subunits. Hbα-A and Hbα-D are different subunits of α-globin found in aquatic and terrestrial turtles, and Hbα-D arose from a gene duplication event of Hbα-A (Hoffmann and Storz 2007). Amino acid sequences differ between the two α-globin subunits which affects the protein's O_2 binding abilities (Perutz 1983).

The many environments that members of the family, *Testudinidae* (tortoises and turtles) inhabit (such as fresh flowing water, stagnant muddy water, and on the land), vary in oxygen levels. The aquatic painted turtle (*Chrysemys picta*) inhabits rivers, lakes and marshes. Lake water and rivers are reliant on oxygen from the air replenishing the oxygen in the water (Donato 2000. The painted turtle can survive 3 to 6 months at 3°C without oxygen (Warren 2006).

Primary protein sequences were determined from the painted turtle and were compared with sequences obtained from GenBank (AB115330; AF304335.1; U63145.1) of the red-footed tortoise (*Geochelone carbonaria*), which is terrestrial and is known for having hemoglobin with low O_2 affinity (Torsoni and Ogo, 1995).

Materials and Methods

- **mRNA Extraction:** Total RNA was was extracted from blood samples from the western painted turtle and African spurred tortoise.
- **RT-PCR:** Reverse transcriptase PCR was done using primers designed for turtle globin genes (Hbα-A, Hbα-D, and Hbb). The PCRs were run on gels to verify successful amplification.
- **Cloning:** Upon successful amplification, hemoglobin alleles were ligated and transformed into competent *E. coli*, and grown on selective media.
- **Colony PCR:** PCR was conducted on the colonies to isolate individual hemoglobin alleles. The PCRs were run on gels to verify successful amplification.
- **Sequencing:** The colonies with inserts of appropriate DNA size were sent off for sequencing at Idaho State Molecular Research Core Facility.
- **Analysis of data:** These preliminary data were analyzed to look for consensus sequences and differences in amino acid composition.

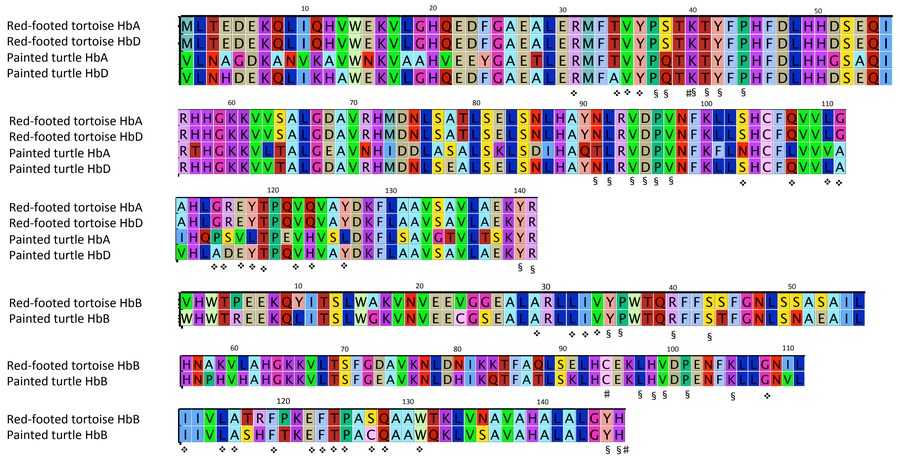


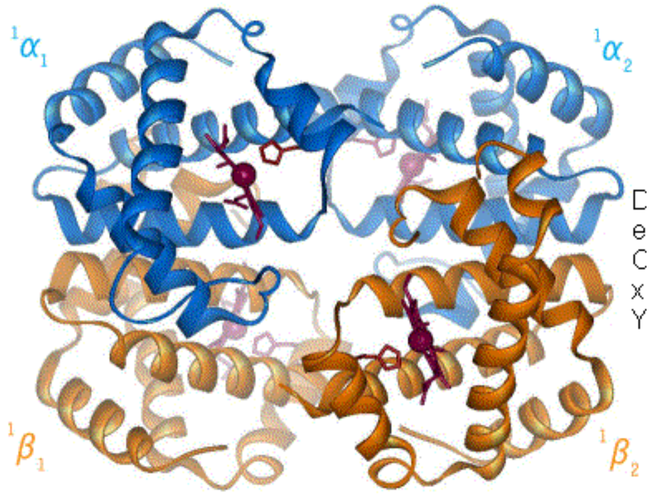
Figure 1. Protein sequence alignment of α-globin (141 aa) and β-globins (146 aa). The symbol ❖ indicates residues involved in contact of subunits α_1 and β_1, and the symbol § indicates residues involved in α_1 β_2 contact. The symbol # indicates amino acids which participate in forming salt bridges that stabilize the deoxy (T) quaternary structure (Bohr Effect).

Results

We recovered three protein sequences from the painted turtle (Hbα-A, Hbα-D, and Hbβ). Unlike the terrestrial red-footed tortoise, the painted turtle's Hbα-A and Hbα-D protein sequences differed at many amino acid residues. Hemoglobin protein variation was also observed between the terrestrial and aquatic species.

Conclusions and Future Work

Certain residues show the contact of α (A and D subunits) and β hemoglobin. Differences of amino acids between the species are critical at these residues because it can result in different oxygen binding abilities. Within multiple residue sites of α-globin, variation may result in different oxygen binding; the variation is shown in the A and D subunits of the painted turtle. Future work will examine additional species, which experience different oxygen conditions. Future work will also examine oxygen binding abilities of the different hemoglobin proteins in order to understand the significance of the protein differences.



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ADAPTIVE CHANGES IN HEMOGLOBIN IN THE AFRICAN SPURRED TORTOISE AND PAINTED TURTLE

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Through evolution, hemoglobin oxygen affinity has been altered in species that live in different environments. Hemoglobin is the blood protein that carries O_2 from the lungs to the tissues, and returns CO_2 (from the tissues) to the lungs. Members of the family Testudinidae (turtles and tortoises) live under different environmental conditions and thus experience different oxygen levels. The hemoglobin of adult turtles is comprised of α-globin (alpha-D and alpha-A) and β-globin. For this research, hemoglobin of the terrestrial African spurred tortoise (*Geochelone sulcata)* and the aquatic painted turtle (*Chrysemys picta*) were analyzed. The African spurred tortoise lives in the hot, arid environments such as the Sahara, while the painted turtle live in calm freshwater environments, with muddy water bottoms. Analyses of the hemoglobin proteins were done through reverse-transcriptase polymerase chain reaction (RT-PCR) using primers designed for turtle globin genes. PCR products were then cloned and grown on selective media, and sequenced. Sequences were translated into amino acids and analyses were conducted to determine if differences in particular amino acid residues could be important in affecting the oxygen affinity of the hemoglobin protein.