

Analysis of Oxygen Affinity of the Major Hemoglobin Component HbA from *Aquila chrysaetos* (Golden Eagle)

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Abstract

Aquila chrysaetos (golden eagle), a high altitude bird is usually found perched on poles or cliff edges. Its hemoglobin is adapted to high altitude hypoxic condition. To understand the mechanism of respiration in *A. chrysaetos* we need to understand the three dimensional structure of its hemoglobin. During our contemplation, a three dimensional model of hemoglobin from *A. chrysaetos* was predicted through homology modeling using Turkey (*Meleagris gallopavo*)'s HbA as template. MODELLER was used to create three dimensional patterns. Stereochemistry of the models was evaluated by PROCHECK and ProSA. This study is about the analysis of effects of inter-subunit contacts on oxygen affinity of the hemoglobin. The effect of the following pairs of inter-subunit contacts on the oxygen affinity of the hemoglobin have been studied *i.e.* $\alpha 99$ and $\beta 101$, $\alpha 34$ and $\beta 125$, $\alpha 38$ and $\beta 99$ and $\beta 97$, $\alpha 119$ and $\beta 55$, $\alpha 35$ and $\beta 128$, and $\alpha 103$ and $\beta 112$. The absence of bonds between these pair like other hypoxic condition dwellers might be the main cause of its living under low oxygen tension.

Keywords: High altitude, hypoxic, Inter-subunit Contacts, R-state, birds

INTRODUCTION

Hemoglobin (Hb)'s structural and functional correlation in animals other than human has been extensively studied (Perutz, 1983, Weber *et al.*, 2004). Previous studies regarding vertebrates hemoglobin functions explain the metabolic needs and confirms that the structure and function is responsible for environmental constraints. The organisms demand for the CO₂ and O₂ is full filled by Hb (Stepuro and Zinchuk, 2006). The variation in the structure and thus in function of this molecule indicates the evolution in the animal kingdom for adaptation (Hourdez and Weber, 2005). The obvious variability in Hb molecule in terms of O₂ affinity in response to change in surrounding has been reported long before (Riggs, 1964). Among vertebrates, birds with exception of flightless birds have acquired the ability of surviving in hypoxic condition such as flying at high altitude during migration or in deep sea diving (Weber, 1995).

Golden Eagle is a high altitude bird, and has been reported from Northern Hemisphere, comprised of Asia continent south to Himalayas and China. These birds are also found in North America, which ranges from northern Alaska south to Lower California and central Mexico (Dawson, 1923). The population of Golden eagles inhabiting at remote areas can be affected by human exploration, and hunting causing disturbance near the nest (Kaufman, 1996, Dawson, 1923). As golden eagle inhabit in high altitude, its survival at high altitude is made possible by some of the substitution in the interaction in Alpha and Beta chain residues in Hb, which enable it to survive under hypoxic condition at high altitude.

MATERIALS AND METHODS

The sequences of αA and β chains of *A. chrysaetos*'s Hb (Oberthur *et al.*, 1983) were retrieved from SwissProt Database (Boeckmann *et al.*, 2003). To perform similarity searches and template selection BLAST (Altschul *et al.*, 1997, Altschul *et al.*, 2005) was used. Turkey oxy-hemoglobin A [PDB: 2QMB] (Charles *et al.*, To be Published) was selected as the template because of its highest homology with the target sequence. The αA chain of *A. chrysaetos*'s HbA shows 87 % identity with αA chain of turkey's HbA and the β chain shows 95 % similarity with the β chain of turkey's HbA. The 3D structure coordinates of turkey's HbA were obtained from Brookhaven Protein Databank (PDB) (Berman *et al.*, 2000). Alignment was performed with CLUSTAL-X (Larkin *et al.*, 2007).

MODELLER 9v9 (Sali and Blundell, 1993) was used to build the homology models using turkey's HbA as template. Stereochemistry of the models was evaluated by PROCHECK (Laskowski *et al.*, 1993). The energy graphs were calculated with the help of ProSA (Sippl, 1993). The best model was selected on the basis of PROCHECK and ProSA results.

To analyze the inter subunit contacts LigPlot (Wallace *et al.*, 1995) was used. All protein structures and models were visualized and analyzed using the DS Visualizer® (v. 2, Accelrys Software Inc).

RESULTS AND DISCUSSION

A homology model of the *A. chrysaetos*'s Hb has been calculated using coordinates of the structure of turkey oxy-hemoglobin. The model has general all alpha topology with no beta strands just like all other hemoglobin having eleven alpha helices in Alpha chain and thirteen helices in Beta chain as illustrated in figure 1.

Analysis of the model of *A. chrysaetos* shows that 93.2% residues were in core region, 6.6% were found in allowed region, and 0.2% in generously allowed region while no residue was in the disallowed region as evaluated by PROCHECK. Energy plots of both the chains were below zero just like corresponding template chains' energy plots calculated using ProSA. The energy values of both the chains are quite similar to the corresponding chains of the template.

Inter-subunit Contacts and Their Effect on Oxygen Affinity

The functional characteristics of hemoglobin are because of the inter-subunit contacts i.e. $\alpha 1\beta 1$ and $\alpha 1\beta 2$ as well as its interaction with effectors molecules like Cl, CO₂ and organic phosphates (Perutz, 1989). In Tufted duck's HbA, the formation of the salt bridge between $\alpha 99\text{Arg}$ and $\beta 101\text{Glu}$ stabilize the R (Relaxed) state that helps in increasing the oxygen affinity of the hemoglobin (Hourdez and Weber, 2005). In golden eagle hemoglobin $\alpha 99$ is replaced with Lys. These two residues are close enough to interact with each other and hence making a bond that may increase the oxygen affinity of golden eagle's Hb, as represented in figure 2.

Most birds, including Bar Headed goose, possess Thr at position $\alpha A34$ ($\alpha 1\beta 1$ contact site). The interaction between $\alpha A34$ and $\beta 125$ results into a hydrogen bond. It stabilizes the T structure and hence lowering the oxygen affinity (Lutfullah *et al.*, 2005). This hydrogen bond is lost in Tufted duck's HbA because of the substitution of Thr with Ile at $\alpha 34$ position (Hourdez and Weber, 2005). This loss of hydrogen bond stabilizes the R structure and hence increasing the oxygen affinity of Tufted duck's HbA. Pheasant's HbA possesses Ile at $\alpha 34$, which cannot make a hydrogen bond with $\beta 125\text{Glu}$ thus having high oxygen affinity (ALI *et al.*, 2011). The golden eagle's HbA has Thr at $\alpha A34$ position but it did not show any interaction with the $\beta 125$ in R state which stabilized the R state and thus may be a possible cause of high oxygen affinity as represented in figure 3.

Anseriformes and some other species have Gln at position $\alpha 38$ (Huber *et al.*, 1988), which is responsible for stable oxy structure of hemoglobin (Perutz, 1990) by making two hydrogen bonds with $\beta 99$ and $\beta 97$. These bonds are also reported in Tufted duck's HbA and thus are the possible agents of its high oxygen affinity (Hourdez and Weber, 2005). In case of Golden eagle $\alpha 38\text{Gln}$ is substituted with Pro, and beta residues are replaced by $\beta 97\text{His}$ and $\beta 99\text{Asp}$. The distance between $\alpha 38\text{Pro}$ and $\beta 97\text{His}$ residues was shorter than that of $\alpha 38\text{Pro}$ and $\beta 99\text{Asp}$ as a result, unlike the Tufted duck's HbA, hydrogen bond was found between $\alpha 38\text{Pro}$ and $\beta 97\text{His}$. The bond was absent between $\alpha 38\text{Pro}$ and $\beta 99\text{Asp}$. The presence of this bond in R state ensures the high oxygen affinity. Figure 4 shows the interaction between these residues.

In Human HbA $\beta 55\text{Met}$ is involved in van der Waals interactions with $\alpha 119\text{Pro}$, however this type of interaction is not possible in Bar Headed goose and Andean goose because of the mutation in this pair of residues i.e. $\beta 55\text{Leu}$ and $\alpha 119\text{Ala}$ in Bar Headed goose, and $\beta 55\text{Ser}$ and $\alpha 119\text{Pro}$ in Andean goose (Hiebl *et al.*, 1987, Oberthur *et al.*, 1982). Due to the absence of this contact in Bar Headed goose and Andean goose HbA, T structure becomes unstable increasing the oxygen affinity (Hourdez and Weber, 2005). In pheasant, the substitution of Leu at position $\beta 55$ fails to show van der Waals interactions with $\alpha 119\text{Pro}$ due to larger distance and results into increased oxygen affinity (ALI *et al.*, 2011). In golden eagle's HbA R-state, $\beta 55\text{Leu}$ is mutated to $\beta 55\text{Ile}$. Due to larger distance between these two residues no van der Waals interactions are observed, which is a possible inclination to lower oxygen affinity, as represented in figure 5.

Some additional contacts which effect the hemoglobin adaptation of Golden eagle

Some interactions between α and β subunits results into stabilization of the T-state as a result more quantity of oxygen needs for relaxation of tense state (Bettati *et al.*, 1998). It has been made clear in previous studies that more inter-subunit contacts result in lower oxygen affinity in the hemoglobin molecules (Liu *et al.*, 2001). Some additional contacts have been reported in previous studies between the *G. carbonaria* Hb results low O₂ affinity relative to chicken and human Hb (Lutfullah *et al.*, 2008). A substitution in human Hb is reported at $\alpha 35$ where Ser is present, same position in *G. carbonaria* Hb is occupied by Val (Lutfullah *et al.*, 2008). In golden eagle Hb's alpha chain, Thr is present at $\alpha 35$ position and do not form hydrogen bond with $\beta 128\text{Leu}$, as illustrated in figure 6, which may possibly increase oxygen affinity of golden eagle's Hb. Similarly, at $\beta 112$, Ile is present in *G. carbonaria* HbD $\beta 112$ is substituted by Ile and differentiate from human having Cys at same position form H-bond with $\alpha 103\text{His}$ (Lutfullah *et al.*, 2008). Just like *G. carbonaria* HbD and unlike human Hb Ile is present at $\beta 112$ but $\alpha 103\text{His}$ is substituted by $\alpha 103\text{Ser}$, due to larger distance they are unable to form bond, figure 7 shows the distance between these residues. This absence of bond may have increased the oxygen affinity in golden eagle's HbA.

Heterotropic effects

The Hb O₂-binding affinity depend on allosteric effectors interactions which may be H⁺ ions, organic phosphates, Cl⁻ and CO₂. They bind strongly to deoxyHb, mainly at sites of N- and C-termini, so stabilized T-state and hence low O₂ affinity by salt bridges formation (Bettati *et al.*, 1983, Perutz, 1970). At normal PH, the human Hb binds

to protons at $\alpha 1\text{Val}$, $\alpha 122\text{His}$, $\beta 2\text{His}$, $\beta 82\text{Lys}$, $\beta 143\text{His}$, and $\beta 146\text{His}$ (Ho and Russu, 1987, Kilmartin *et al.*, 1978, Lukin and Ho, 2004, Perutz *et al.*, 1969). Cl^- ions binds to $\alpha 1\text{Val}$ and $\alpha 131\text{Ser}$ and $\beta 1\text{Val}$ and $\beta 82\text{Lys}$ (Riggs, 1988). While the CO_2 combines with the N-terminal NH_3^+ residues of deoxyHb and change the O_2 affinity through delocalized electrostatic effects (Arnone, 1974, Perutz, 1983, Perutz *et al.*, 1994). The positive charges of β -chains of deoxyHb is partially neutralized by Chloride ions and stabilizes the deoxy state. Similar to other vertebrates the above residues are conserved in golden eagle except $\beta 143\text{His}$ which is substituted by $\beta 143\text{Arg}$, which may be the possible reason for the increased oxygen affinity and ability of surviving in lower oxygen pressure.

The joint effects of hypoxia and cold in high-altitude habitats compel to intense physiological challenges on endothermic animals. At high altitude the low oxygen pressure decreases the oxygen distribution arriving at the cells of respiring tissues (Chevion and Brumfield, 2012). As golden eagle is a high altitude bird, it overcome these entire problems by the unique interactions in the Hb inter subunits contacts. It is concluded from the above study that the HbA of golden eagle has been adapted to hypoxic condition.

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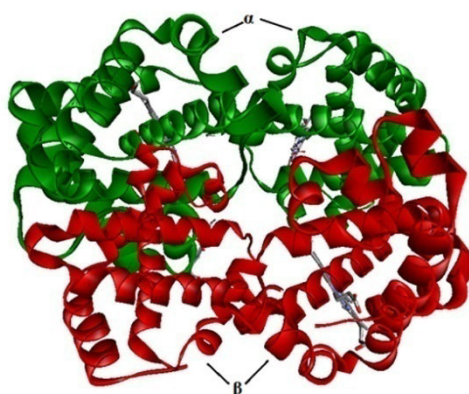


Figure 1: Schematic representation of the predicted homology model of *Aquila chrysaetos*'sHbA. Hem is represented in ball and stick representation whereas globin chains are shown as flat ribbons (Alpha chains: green and Beta chains: yellow)

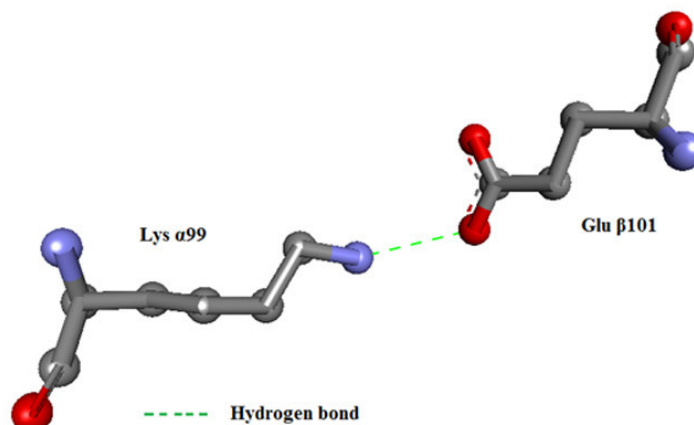


Figure 2: The hydrogen bond between $\alpha 99$ Lys and $\beta 101$ Glu. The residues have been represented with ball and stick model and the hydrogen bond is shown by dotted line.

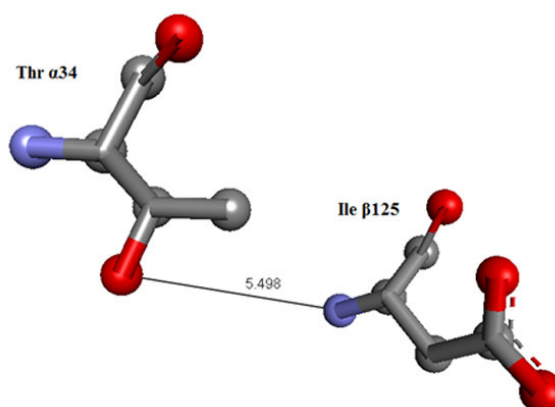


Figure 3: The distance between $\alpha 34$ Thr and $\beta 125$ Ile. The residues have been represented with ball and stick model and the distance is shown with a line.

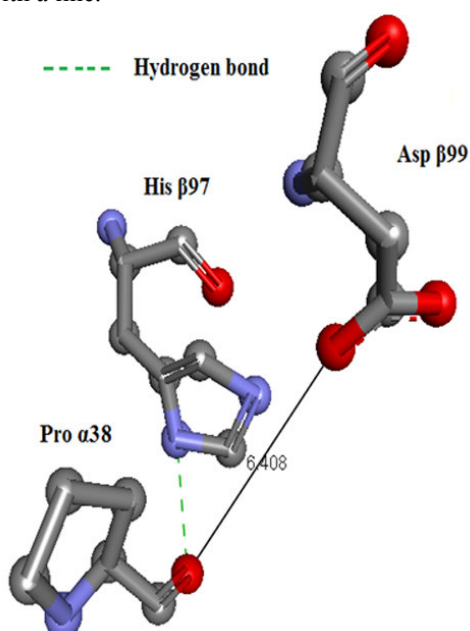


Figure 4: The distance between α Pro38, β 1Asp99 is shown by a line and the hydrogen bond between α 1Pro38, β 1His97 is represented by dotted line.

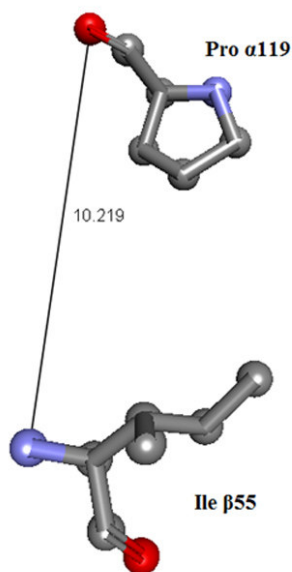


Figure 5: The representation of the distance between α Pro119 and β Ile55.

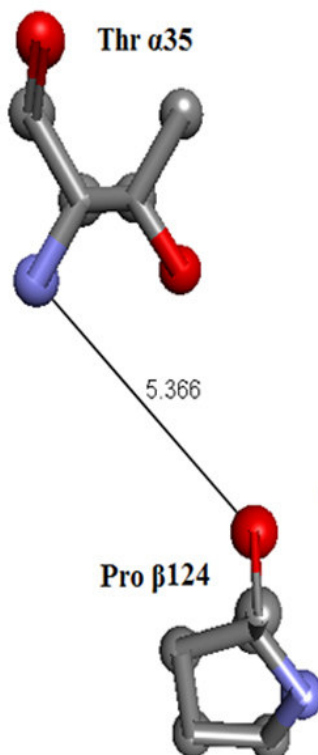


Figure 6: The distance between α 35Thr and β 124Pro. The residues have been represented with ball and stick model and the distance is shown with a line.

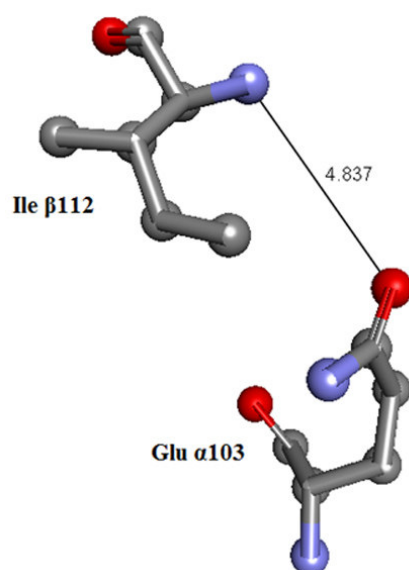


Figure 7: The residues α 103Glu and β 112Ile have been represented with ball and stick model and the distances have been shown with lines.

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