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Analysis of Oxygen Affinity in Aquatic Amphibian - Homology Modeling of the Major Hemoglobin Component HbA from *Ambystoma mexicanum*

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ABSTRACT

Ambystoma mexicanum an obligate paedomorphic species has large number of substitution in the interaction in Alpha and Beta chain residues of its hemoglobin that enable it to survive in hypoxic and lower oxygen pressure condition. To understand the mechanism of respiration in *A. mexicanum* we need to understand the three dimensional structure of its hemoglobin. In this study, we have built a three dimensional homology model of hemoglobin A (HbA) from *A. mexicanum* using bovine HbA as template. MODELLER was used to create three dimensional patterns and was evaluated with ProSA and PROCHECK. 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.* α99 and β101, α34 and β125, α38 and β99 and β97, α119and β55, α35 and β128, and α103 and β112. It has been predicted from the loss of interactions between these pairs of residues that *A. mexicanum* HbA might be able to tolerate hypoxic conditions and have greater oxygen affinity.

Keywords: *Ambystoma mexicanum,* oxygen affinity, hypoxic state, inter sub unit contacts

INTRODUCTION

The metabolic needs of vertebrates are met by hemoglobin functional properties, and its functions determine organism survival in a particular habitat [1]. Hemoglobin diversity shows its evolution in each phyla for adaptation and survival [2]. Hemoglobin shows variation for oxygen affinity and its susceptibility to modulation by metabolic effectors in its environment [3].

The Amphibia appeared to be an interesting class for hemoglobin studies. Within this class are found aquatic, semi aquatic and terrestrial types: i.e. varying degrees of transition from aquatic to terrestrial habitat. Here the three primary loci of erythrocyte formation are functional [4]. *Ambystoma mexicanum* is an obligate paedomorphic species, endemic to the valley of Mexico. It is widely used as model organism in evolutionary and developmental biology and thus commonly maintained in captivity, with several breeding colonies around the world [5]. The large number of substitution in the interaction in Alpha and Beta chain residues in *A. mexicanum* enable it to survive in hypoxic and lower oxygen pressure condition.

MATERIALS AND METHODS

Primary Sequence Analysis

The sequences of αA and β chains of *A. mexicanum* HBA [6] were retrieved from SwissProt Database [7]. To perform similarity searches and template selection BLAST [8, 9] was used. Bovine hemoglobin A (PDB ID: 1HDA) [10] was selected as the template because of its highest homology with the target sequence. The αA chain of *A. mexicanum* HBA shows 51 % identity with αA chain of bovine HbA and the β chain shows 52 % similarity with the β chain of bovine HbA. The 3D structure coordinates of bovine HbA were obtained

from Brookhaven Protein Databank (PDB) [11]. Alignment was performed with CLUSTAL-X [12].

Model Building and Evaluation

MODELLER 9v9 [13] was used to build the homology models using bovine HbA as template. Stereochemistry of the models was evaluated by PROCHECK [14]. The energy graphs were calculated with the help of ProSA [15]. The best model (Fig.1) was selected on the basis of PROCHECK and ProSA results. To analyze the inter subunit contacts LigPlot [16] was used. Protein structures were analyzed and visualized through DS Visualizer® (v. 2, Accelrys Software Inc).

Results and Discussion

A homology model of the *A.mexicanum* HbA (Fig. 1) has been calculated using coordinates of the structure of bovine deoxyhaemoglobin [10]. The model has general all alpha topology with no beta strands just like all other hemoglobin having seven α helices in α chain and eight helices in β chain.

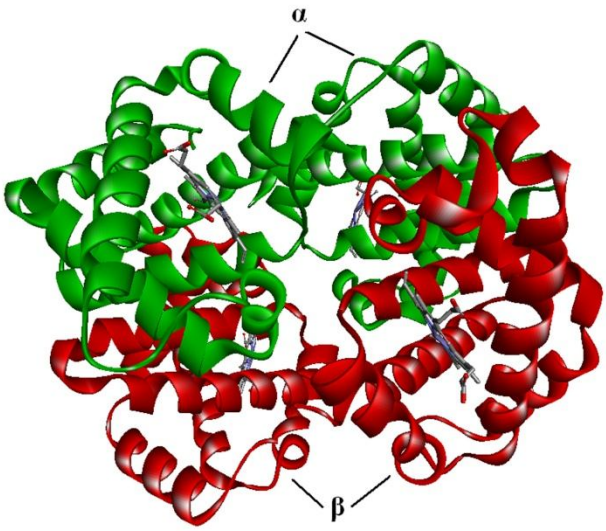

Figure 1. Schematic representation of the predicted homology model of *A. mexicanum* HBA. Hem is represented in ball and stick representation whereas globin chains are shown as flat ribbons (Alpha chains: Green and Beta chains: Red).

Analysis of the model of *A.mexicanum* shows that 95.2% residues were found in core region, 3.8% in additionally allowed region, and 1.0% in generously allowed region while no residue was found in the disallowed region (Fig. 2) 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 derived from inter-subunit contacts i.e. α1β1 and α1β2 as well as its interaction with effectors molecules like Cl, CO2 and organic phosphates [17]. In Tufted duck's HbA, the R (Relaxed) structure is stabilized by a salt bridge between α99Arg and β101Glu that helps in increasing the oxygen affinity of the hemoglobin [2] while in pheasant's HbA (deoxy state) at α1β1 contact site α99Arg is replaced with α99Lys, which cannot make salt bridge [18], making the T state unstable, hence increasing the oxygen affinity. Similarly in *A.mexicanum* HbA at α1β1 contact site α99Pro is present, which do not form salt bridge in its T-state, because β101Glu is replaced with β101Gln which is an uncharged amino acid in nature (Fig. 3).This loss of salt bridge in T-state might increase the oxygen affinity of *A. mexicanum* HbA by destabilizing the T-state.

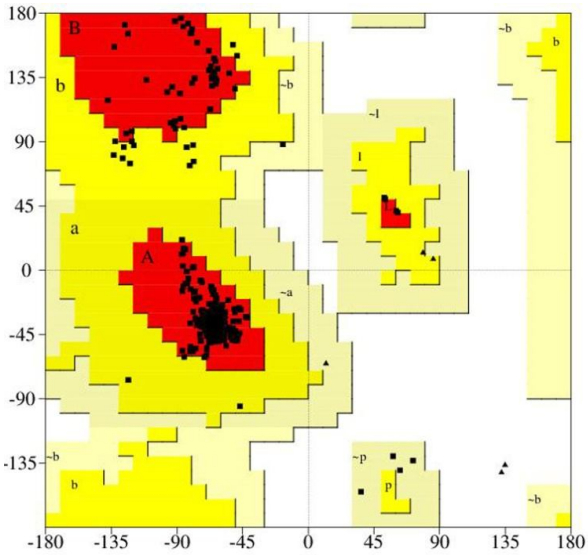

Figure 2. Ramachandran plot of *A. mexicanum* HBA homology model.

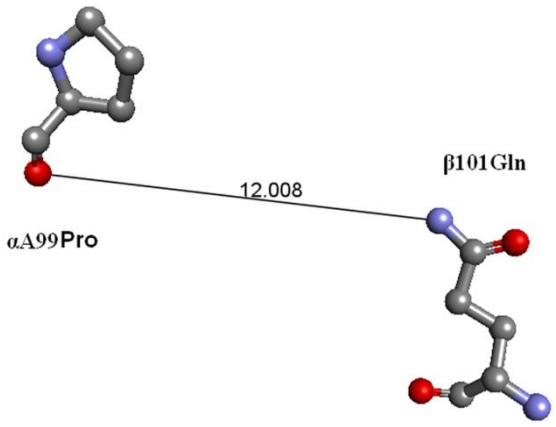

Figure 3. The distance between αA99Pro and β101Gln. The residues have been represented with ball and stick model and the distance has been shown with a black line.

Most birds, including Bar Headed goose, possess Thr at position αA34 (α1β1 contact site). The interaction between αA34 and β125 results into a hydrogen bond. It stabilizes the T structure and hence lowering the oxygen affinity [19]. This hydrogen bond is lost in Tufted duck's HbA because it has been replaced with Ile at α34 position [2]. This loss of hydrogen bond stabilizes the R structure and hence increases the oxygen affinity of Tufted duck's HbA. Pheasant's HbA

possess Ile at α34, which cannot make a hydrogen bond with β125Glu thus having high oxygen affinity [18]. *A. mexicanum* HbA have Thr at α34 and His at β125. Although first one is uncharged polar and second one is basic, but are unable to make any bond in T-state, because of the greater distance between them (Fig. 4). Just like the Tufted duck's HbA and pheasant's HbA Loss of this bond can stabilize the R structure of *A. mexicanum* HbA which might raise the oxygen affinity of its hemoglobin.

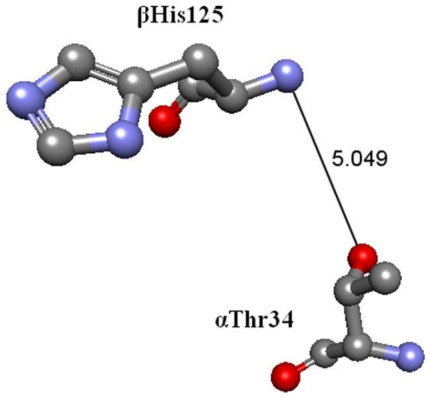

Figure 4. Ball and stick model representation of αThr34 and βHis125.

Anseriformes and some other species possess Gln at position α38 [20], which is responsible for stable oxy structure of hemoglobin [21] by making two hydrogen bonds with β99 and β97. Tufted duck's HbA possess higher oxygen affinity due to these two hydrogen bonds [2].In Pheasant's HbA, Gln is substituted by Ser at this position, which, due to its smaller size and orientation (larger distance between them), is unable to make hydrogen bonds with β99Asp and β97His, destabilizing the T-state, hence increasing the oxygen affinity [18]. In case of *A. mexicanum* Gln α38 is present just like the Pheasant's HbA , due to the greater distance between Gln α38 and beta residues(β97Phe and β99Asp) do not interact to form hydrogen bond in T-state, which destabilize the T-state and might stabilize the R-state (Fig. 5). As a result the oxygen affinity of *A .mexicanum* HbA can be higher.

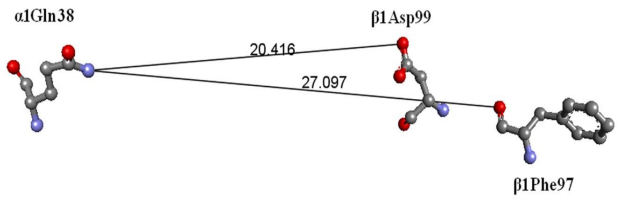

Figure 5. The distance between α1Gln38, β1Phe97 and α1Gln38, β1Asp99. The black lines indicate the distance between alpha and beta subunit residues.

In Human HbA β55Met is involved in van der Waals interactions with αPro119, however this type of interaction is not possible in Bar Headed goose and Andean goose because this pair of residues is mutated to smaller residues, i.e. β55Leu and α119Ala in Bar Headed goose, and β55Ser and α119Pro in Andean goose [22, 23]. Due to the absence of this contact in Bar Headed goose and Andean goose HbA, T structure becomes unstable increasing the oxygen affinity [2]. In pheasant, uncharged residue Leu is substituted at position β55 which cannot make van der Waals interactions with α119Pro due to larger distance, thus increasing the oxygen afiinity[18]. In *A. mexicanum* T-state, both β55Leu and α119Pro are mutated to β55Cys and α119Tyr, which are uncharged polar, thus no van der Waals interactions are observed because of larger distance(Fig. 6), which might be a possible cause of increased oxygen affinity.

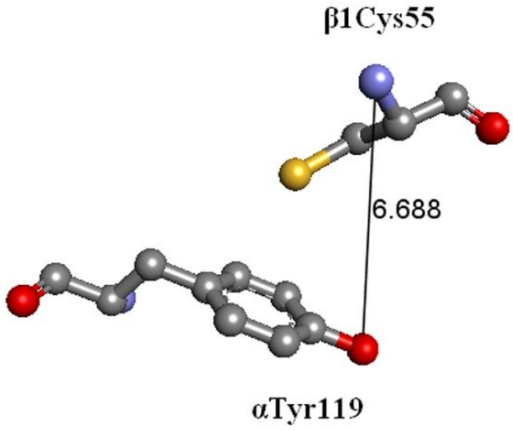

Figure 6. The observed distance between αTyr119 and β1Cys55 of *A. mexicanum* HBA.

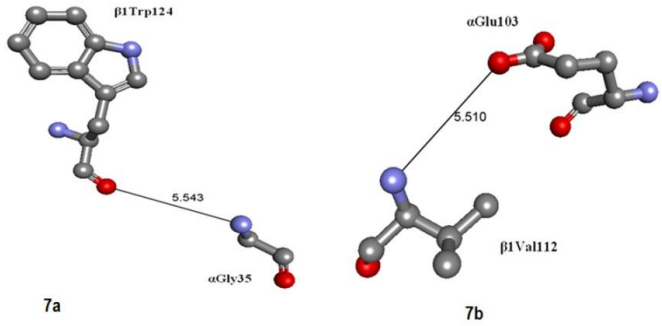

Figure 7a, b. Illustrates the distance between alpha and beta residues having no hydrogen bond.

Some additional contacts which effect the hemoglobin adaptation of *A. mexicanum*

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 [24]. It has been made clear in previous studies that more inter-subunit contacts result in lower oxygen affinity in the hemoglobin molecules [25]. Some additional contacts have been reported in previous studies between the *G. carbonaria* Hb results low O2 affinity relative to chicken and human Hb [26]. A substitution in human Hb is reported at α35 where Ser is present, same position in *G. carbonaria* Hb is occupied by Val. In *A. mexicanum* alpha chain, Gly is present at α35 position and do not form hydrogen bond with β128Leu in T-state of HbA (Fig. 7a), which may possibly increase oxygen affinity of HbA. Similarly, at

β112, Ile is present in *G. carbonaria* HbD β112 is substituted by Ile and differed from human having Cys at same position form H-bond with α103His [26]. In the T-state of *A. mexicanum* no such contact (Fig. 7b) is found except the substitution of β112Cys (in human) to β112Val (*A. mexicanum*), which cannot stabilize the T-state of *A. mexicanum* and thus HbA may increase the oxygen affinity.

Hemoglobin and Allosteric Effectors

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 [27, 28]. At normal PH, the human Hb binds to protons at α1Val, α122His, β2His, β82Lys, β143His, and β146His [29-32]. Cl^- ions binds to α1Val and α131Ser and β1Val and β82Lys [33], While the CO2 combines with the N-terminal NH3+ residues of deoxyHb and change the O2 affinity through delocalized electrostatic effects [34, 35]. It has also been hypothesized that Cl^- may modulate O2 affinity through delocalized electrostatic effects that do not involve binding at specific residues [36]. The positive charges of β-chains of deoxyHb are partially neutralized by Chloride ions and stabilize the deoxy state. Similar to other vertebrates the above residues are conserved in golden eagle except β143His which is substituted by β143Arg, which may be the possible reason for the increased oxygen affinity and ability of surviving in lower oxygen pressure. Similar to other vertebrates the above residues are conserved in *A. mexicanum* except α131Ser which is substituted by α131Val, which may be the possible reason for the increased oxygen affinity and ability of surviving in lower oxygen pressure.

CONCLUSION

The effect of inter-subunit contacts on the oxygen affinity of the hemoglobin have been studied *i.e.* α99 and β101, α34 and β125, α38 and β99 and β97, α119and β55, α35 and β128, and α103 and β112. The loss of interaction between these residues is responsible for oxygen affinity of an organism. It has been predicted from the loss of interactions between these pairs of residues that *A. mexicanum* HbA might be able to tolerate hypoxic conditions and have greater oxygen affinity at T-state.

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