Analysis of oxygen affinity in aquatic amphibian; homology modelling of the major Haemoglobin component HbA1 from the African clawed frog (*Xenopus laevis*, Anura)

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Abstract: The homology model of major haemoglobin component HbA1 of the African Clawed Frog was predicted using the pigeon (*Columba livia*) haemoglobin as a template. The model was built with the help of MODELLER9v8. The models were evaluated with ProSA and PROCHECK. In *X. laevis* Gln38α is unable to form a hydrogen bond with β97His or β99Asp, which is responsible for the increase in oxygen affinity of the Xenopus HbA1. The hydrogen bond between α34Thr and β124Pro, which stabilises the deoxy state of the haemoglobin, was absent in *X. laevis*. Hence it is predicted that the HbA1 component of *X. laevis* has higher oxygen affinity.

Keywords: *Xenopus laevis*; haemoglobin; homology modelling; amphibia; high oxygen affinity; aquatic.

1 Introduction

Haemoglobin, a widely distributed protein molecule that stores oxygen, is unique in adaptability to different environmental conditions (Hardison, 1998). Haemoglobin which is a tetrameric proteinous molecule has a heme molecule attached to each subunit. Oxygen, in the presence of ligands and effectors molecules, binds cooperatively with haemoglobin (Perutz, 1989).

The oxygen affinity of an animal’s haemoglobin depends upon its natural habitat. Haemoglobin of certain native high altitude mammals and birds has a significantly greater affinity for oxygen than those of members of their class at low level (Dill et al., 1963). Among vertebrates, birds have acquired higher oxygen affinity (Mairbaurl, 1994) to adapt to a wide variety of environmental conditions. Avian haemoglobin has efficient oxygen transport characteristics leading it to cope with the severe hypoxic conditions, an important adaptation contributing to exceptional tolerance at extreme altitudes and has successfully survived the evolutionary pressures (Weber, 1995).

Unlike other vertebrates, members of the class amphibians utilise every type of respiratory gas exchange known in the vertebrates i.e., gills, lungs, skin, and buccopharyngeal mucosa, different species using different combinations depending on their natural environment. Within Amphibians are found aquatic, semi-aquatic and terrestrial inhabitants (Noble and Putnam, 1931; Hall, 1966; McCutcheon and Hall, 1937;
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Walter and Victor, 1965). *Xenopus laevis* (X. Laevis) is one of the examples of aquatic amphibian. As deep sea or aquatic animals need greater oxygen to be stored in their blood therefore they have greater hematocrits (Hillman, 1976). To cope with its aquatic environment, it is necessary that their haemoglobin must have higher oxygen affinity. In this study the haemoglobin of *X. laevis* was selected as model for the study of oxygen affinity in aquatic amphibians because it is highly aquatic and is widely used as model organism in scientific research.

*X. laevis* is included in order Anura, which is a large, and diverse group comprising more than 5,400 species (http://www.britannica.com/EBchecked/topic/29023/Anura#: retrieved on November 10, 2010). They have a flattened head and body, but no tongue or external ears (Garvey, 2000). The species is found throughout most of Africa, and in isolated, introduced populations in North America, South America, and Europe (Nieu koop and Faber, 1994). These frogs are plentiful in ponds and rivers within the south-eastern portion of Sub-Saharan Africa. They are aquatic and are often greenish-grey in colour (Garvey, 2000; Nieu koop and Faber, 1994). Albino-varieties are commonly sold as pets. The blood of adult *X. laevis* contains six electrophoretically resolvable globin chains. Xenopus tadpoles’ blood has two haemoglobin components i.e., HbF1 and HbF2, while the adult Xenopus blood contains two adult haemoglobin components i.e., HbA1 and HbA2 with some traces of tadpole haemoglobin. HbA1 comprises the majority of the adult haemoglobin (Maclean and Jurd, 1971; Hentschel et al., 1979).

In this study, we have predicted the three dimensional homology model of HbA1 tetramer of *X. laevis* because the crystal structure was not resolved at the time of this research work. The present study provided information about the functional implications of various structural features in HbA1 and its correlation with the oxygen affinity.

2 Results and discussion

2.1 Homology model of *Xenopus laevis* HbA1

The HbA1 homology model of *X. laevis* has 574 amino acid residues excluding the starting methionine residue of both the alpha chains. Pairwise sequence alignment of *X. laevis* α and β chains with the corresponding chains of pigeon HbA is shown in Figure 1(a) and (b) respectively. A heme group is attached to each of the four chains of the HbA1 tetramer in its active site (Figure 2). Model has the same general topology of the template. Like other HbA molecules the heme contact residues are well conserved (Figure 3).

Ramachandran plot of the homology model of *X. laevis* HbA1 shows that 94.3% residues are in allowed, 5.1% in additionally allowed and 0.6% residues are in the generously allowed regions while no residue was found in the disallowed region (Figure 4(a)). These values are almost similar to Ramachandran plot values for the pigeon HbA taken as template (Figure 4(b)). ProSA energy plots of the model are well below the zero. For overall topological analysis the Ca backbone of the predicted model was superimposed on the template structure (Figure 5). The model superimposed well over the pigeon HbA. The root mean square deviation was calculated to be 0.32 Å. This value is due to the good sequence similarity between the sequences of the two molecules. All, the high similarity between the Ramachandran plots of the template and the model, the
highest number of residues in the core region with no residues in the disallowed region, the good superimposition of the model over the template, and the below zero energy values of the ProSA energy plots show that the model is quite accurately built and is quite reliable.

Figure 1  Pairwise sequence alignment of: (a) *X. laevis* αA1 chain with pigeon αA chain of HbA (3DHR) with consensus sequence (X represent Un-conserved). Conserved residues have been highlighted and (b) *X. laevis* β chain with pigeon β chain of HbA (3DHR) with consensus sequence (X represent Un-conserved). Conserved residues have been highlighted (see online version for colours)

Figure 2  Solid ribbon representation of the *X. laevis* HbA1 model. Heme residues are given in ball and stick. Each chain has been coloured differently (see online version for colours)
2.2 Intersubunit contacts and oxygen affinity

The \( \alpha_1 \beta_1 \) and \( \alpha_1 \beta_2 \) contact residues are almost the same as found in pigeon and other species except a few changes that have been discussed (Figure 6(a) and (b)). The oxygen affinity of haemoglobin is greatly influenced by the alteration of functionally important residues forming salt bridges, H-bonds and other van der Waals interactions.

Figure 3  Heme binding residues as calculated by LIGPLOT (see online version for colours)

The oxygen affinity of the haemoglobin is strongly influenced by the alteration of functionally most important residues at positions \( \alpha_34 \) and \( \alpha_38 \). In human haemoglobin the residue at position \( \alpha_34 \) interacts with \( \beta_{124}, \beta_{125} \) and \( \beta_{128} \) in the oxy structure. However most birds posses Thr at this position, forming hydrogen bond with Glu at \( \beta_{125} \) like chicken, which stabilises the T structure thereby lowering the oxygen affinity (Lutfullah et al., 2005). Our results show that in \( X. \) laevis \( \alpha_34 \) (\( \alpha_35 \), when starting methionine is included) Ile is present which cannot form a hydrogen bond with \( \beta_{124} \)Pro or \( \beta_{125} \)Glu (in T-structure), however only weak van der Waals interactions are present in between them (Figure 7). This type of situation is found in Tufted duck also, where \( \alpha_34 \)Val cannot make a hydrogen bond with the above mentioned \( \beta \) residues resulting in the increase of oxygen affinity. From this it can be predicted that this loss of hydrogen bond might also increase the oxygen affinity of the \( X. \) laevis haemoglobin.

Another functionally important residue influencing the oxygen affinity is at \( \alpha_38 \). Most of the diving (Huber et al., 1988) and high altitude (Hiebl et al., 1988) birds have glutamine at this position including Tufted duck and chicken HbDs (Huber et al., 1988). \( \alpha_38 \)Gln forms two hydrogen bonds with \( \beta_{97} \) and \( \beta_{99} \) in the oxyhaemoglobin HbD of Tufted duck stabilising the \( \alpha_1 \beta_2 \) interface thereby increasing its oxygen affinity (Lutfullah et al., 2005). In the predicted model of deoxyhaemoglobin of \( X. \) laevis, \( \alpha_38 \)Lys is not able to form hydrogen bond with \( \beta_{97} \)His or \( \beta_{99} \)Asp (Figure 8).
destabilising the T-state and hence increasing the oxygen affinity. The absence of hydrogen bonds with β97 and β99 in deoxy state may be involved in the increasing of oxygen affinity of the HbA1 of *X. laevis*.

**Figure 4(a)** Ramachandran plot of the predicted model (Model No. 10) of HbA1 of *X. Laevis* (see online version for colours)

![Ramachandran Plot](image)

**Plot statistics**

- Residues in most favoured regions (A,B,L) 481 (94.3%)
- Residues in additional allowed regions (A,B,L) 26 (5.1%)
- Residues in generously allowed regions [-a,b,-l,-p] 3 (0.6%)
- Residues in disallowed regions 0 (0.0%)
- Number of non-glycine and non-proline residues 510 (100.0%)
- Number of end-residues (excl. Gly and Pro) 8
- Number of glycine residues (shown as triangles) 36
- Number of proline residues 20
- Total number of residues 574

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.
Figure 4(b) Ramachandran plot of the template of pigeon HbA (PDB code: 3DHR) (see online version for colours)

Figure 5 Superimposition of Cα backbone of the predicted model of *X. laevis* HbA1 (violet) over the pigeon HbA (blue) taken as template (see online version for colours)
Figure 6(a) Amino acid residues at α₁β₁ interface of HbA1 of *X. laevis* haemoglobin. The residues of alpha chain are shown in blue, while the beta chain residues are shown in violet. All the residues have been shown in stick representation. Hydrogen bonds have been represented with dotted lines in black colour and have been encircled. Image was created with DS visualiser and edited with Paint.Net program of windows (see online version for colours)

Figure 6(b) Amino acid residues at α₁β₂ interface of HbA1 of *X. laevis* haemoglobin. The residues of alpha chain are shown in blue, while the beta chain residues are shown in violet. All the residues have been shown in stick representation. Hydrogen bonds have been represented with dotted lines in black colour and have been encircled. Image was created with DS visualiser and edited with Paint.Net program of windows (see online version for colours)
In human and chicken, α119Pro forms a hydrogen bond with β55Met (Human) or β55Leu (Chicken) while there is no such bond in Bar headed goose and Andean goose, making it suitable for hypoxic conditions (Liang et al., 2001). The presence of this bond lowers the oxygen affinity of chicken haemoglobin (Lutfullah et al., 2008). Like Bar headed goose and Andean goose (The high altitude birds that can live under hypoxic conditions), the predicted model of *X. laevis* HbA1 does not show a hydrogen bond between α119Pro and β55Phe (Figure 9), hence reaching to a conclusion that HbA1 of *X. laevis* has higher oxygen affinity which is a need of its aquatic nature.
Like chicken and Tufted duck HbD, the predicted model also shows a hydrogen bond between α94Asp and β102Asn (Figure 10). Similar to Tufted duck haemoglobin, α97Asn does not form a hydrogen bond with β99Asp (Figure 11), whereas this bond is present in human haemoglobin (Lutfullah et al., 2005). From this it is concluded that *X. laevis* HbA1 has characteristics similar to the Tufted duck HbD which has high oxygen affinity (Lutfullah et al., 2005).

**Figure 9**  The Hydrogen bonds and van der Waals interactions that β55Phe of *X. laevis* HbA1 can make with nearby residues

**Figure 10**  The Hydrogen bonds and van der Waals interactions that α94Asp of *X. laevis* HbA1 can make with nearby residues
3 Experimental

3.1 Primary sequence analysis

The sequences of α and β chains of HbA1 were retrieved from SwissProt Data resource (Boeckmann et al., 2003). BLAST (Altschul et al., 1997) search was used to find out the template for homology modelling. Pigeon HbA (*Columba livia*, PDB ID: 3DHR) (Sathya Moorthy et al., 2009) was selected as the best template because of its highest homology with the target sequence (Figure 1 (a) and (b)). The α and β chain of *X. laevis* HbA1 shows 59% and 53% identity with the α and β chain of pigeon HbA respectively. The 3D structure coordinates of pigeon HbA were obtained from Brookhaven Protein Databank (PDB) (Berman et al., 2002). Sequence alignment was done with CLUSTAL X (Thompson et al., 1997; Larkin et al., 2007).

3.2 Model building and evaluation

Thirty homology models of *X. laevis* were built by MODELLER 9v8 (Eswar et al., 2008). Stereochemistry of the models was evaluated by using PROCHECK (Laskowski et al., 1993). The energy graphs were calculated using ProSA (Sippl, 1993). The best model (Figure 2) was selected on the basis of PROCHECK and ProSA results. LigPlot (Wallace et al., 1995) was used to analyse the interactions of functionally important residues with the neighbouring chain residues. In order to examine any alteration in the Cα backbone of *Xenopus* HbA1, it was superimposed onto HbA crystal structure of pigeon using the superimposition command of DS Visualiser® (v. 2, Accelrys Software Inc). Root Mean Square Deviation (RMSD) value was calculated using the RMSD command of the same software. All protein structures and models were visualised and analysed using the DS Visualiser® (v. 2, Accelrys Software Inc.).
4 Conclusion

The homology model of *X. laevis* has similar topology as that of pigeon haemoglobin crystal structure. From this study it has been concluded that HbA1 component of *X. laevis* haemoglobin has higher oxygen affinity that makes this species adaptable for its natural aquatic environment.

References


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