New Sequential Model for Human Hemoglobin: Alpha Subunit as Cooperativity Inducer

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ABSTRACT

Hemoglobin is a tetrameric oxygen transport protein in animal bodies. However, there is a paucity of information regarding differences between alpha and beta subunits of hemoglobin in terms of oxygen affinity. The sequential model of Koshland, Nemthy and Filmer (KNF model) has attributed similar affinities to both alpha and beta subunits. The main purpose of the present study is to construct a new sequential model for hemoglobin oxygenation based on higher oxygen affinities for alpha subunits. To this end, coordinate files of 19 oxy and 41 deoxy hemoglobin structures were used as starting structures. These files were processed using Microsoft Excel and SPSS software in order to calculate Euclidean distances between each pair of proximal and distal histidine Fe^{2+} as well as other pairs of atoms of interest. The calculated distances were then compared for either set of hemoglobin conformations, *i.e.* oxy and deoxy conformations. Our results showed that α_2 subunit show higher structural changes that could be related to oxygen affinity. This subunit could be introduced as initiator of hemoglobin oxygenation and cooperativity. Subunit α_2 in our sequential model induces relaxed conformation in α_1, β_2 and β_1 respectively. The order of oxygen affinity in our model is as follow: $\alpha_2 > \alpha_1 > \beta_1 > \beta_2$.

INTRODUCTION

Human hemoglobin is a tetrameric protein consists of two alpha and two beta subunits ($\alpha_2\beta_2$) which carries oxygen from lung to tissues. Hemoglobin binds oxygen reversibly through cooperative contribution of its four subunits [Monod *et al.*, 1965, Changeux & Edelstein, 2005]. Several models have been proposed to explain this cooperativity and its underling molecular events. As per the concerted model of Monod, Wyman and Changeux (MWC model), each subunit of hemoglobin can exist either in R (relaxed) conformation of a higher oxygen affinity and/or in T (tens) conformation of a lower affinity [Rivetti *et al.*, 1993]. Therefore, all subunits must be in T state when the hemoglobin is fully deoxygenated. Once oxygen binds to one subunit, the conformation of all subunits is compelled simultaneously to be in R state [Rivetti *et al.*, 1993, Mozzarelli *et al.*, 1991]. Whereas, the sequential model of Koshland, Nemthy and Filmer (KNF model), suggests that

when oxygen binds to one subunit, it changes only the later conformation to R state. The subunit then induces conformational changes into neighboring residues. There is no constraint for any of subunits to exist in similar conformation [Koshland *et al.*, 1966, Eaton *et al.*, 1999]. As per this model, the induction of conformational change from T to R state in neighboring subunits increases their oxygen affinity to 1-2.5 folds [Eaton *et al.*, 1999, Gill *et al.*, 1986]. In both models, the cooperativity begins with oxygen binding to Fe^{2+}. This binding is followed by displacement of Fe^{2+}-proximal histidine toward heme ring. The movement of proximal histidine is reflected on $\alpha_1\beta_2$ and $\alpha_1\beta_1$ interfaces through protein backbone and leads to breakdown of some non-covalent inter-subunit salt bridge interactions. These alterations, lead to conversion of deoxy conformation of hemoglobin to oxy conformation [Bettati *et al.*, 1998, Mozzarelli *et al.*, 1997].

Despite the fact that the intrinsic oxygen affinity of alpha subunits has been shown to be 4-5 folds higher than that of beta subunits [Bettati *et al.*, 1998, Mozzarelli *et al.*, 1997, Maréchal *et al.*, 2006], nobody has suggested alpha

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subunits as to be responsible for initiating cooperativity upon oxygenation. The major aim of the present work is to build a new sequential model for hemoglobin oxygenation based on preferential binding of oxygen to alpha subunits. To this end, crystal structures of hemoglobin in oxy and deoxy conformations were used to pool structural data which may represent a real model of hemoglobin. The selected coordinate files comprised valuable structural characterization of oxy and deoxy human hemoglobin to serve the purpose of this work.

MATERIALS AND METHODS

Crystal structures of 19 hemoglobin in oxy conformation and 41 in deoxy conformation were used to build a real model of hemoglobin for this study. Coordinate files of these structures were downloaded from Protein Data Bank (http://www.rcsb.org/pdb).

The coordination files of oxy hemoglobin which were analyzed bear the following PDB IDs: 1BBB, 1GZX, 1Y8W, 1YDZ, 1YE0, 1YE1, 1YE2, 1YEN, 1YEO, 1YEQ, 1YEU, 1YEV, 1YG5, 1YGF, 1YH9, 1YHE, 1YHR, 1YIE, 1YIH, and of deoxy hemoglobin with PDBID of: 1A3N, 1BIJ, 1BZ0, 1CLS, 1DXT, 1DXU, 1DXV, 1G9V, 1KD2, 1R1Y, 1RQ3, 1XY0, 1XZ2, 1XZ4, 1Y09, 1Y22, 1Y31, 1Y35, 1Y45, 1Y46, 1Y4G, 1Y4P, 1Y4Q, 1Y4R, 1Y4V, 1Y5F, 1Y5J, 1Y5K, 1Y7C, 1Y7D, 1Y7G, 1Y7Z, 1Y83, 1Y85, 2D60, 2DXM, 2HHB, 3DUT, 3HHB, 4HHB and 6HBW. These files were analyzed to calculate inter-atomic distance using Microsoft Excel. Also, statistical calculations were performed using the Statistical Package for the Social Science (SPSS-PC, version 15. SPSS, Inc., Chicago, IL). Euclidean distances d(i,j) between atom i and j with x, y and z coordinates were measured using the following equation and used as the distance between atoms throughout this study.

$$d(i, j) = \sqrt{(x_i - x_j)^2 + (y_i - y_j)^2 + (z_i - z_j)^2}$$

RESULTS

Figure 1 shows the distance of proximal histidine to ferrous ion (Fe^{2+}) in four subunits of hemoglobin. The average distance calculated for alpha subunits in deoxy

conformation was 2.41 ± 0.02 angstrom. This distance decreased significantly to 2.36 ± 0.02 when alpha subunits were oxygenated (p-value < 0.05). The same distance was, also, calculated for beta subunits in deoxy conformation to be 2.28 ± 0.02 angstrom, but with no significant alteration in this distance when subunits were oxygenated.

Similarly, the distance of distal histidine to Fe^{2+} was calculated for hemoglobin subunits and illustrated in Fig. 2. As depicted, the calculated distance has shown similar pattern of fluctuation for both alpha and beta subunits emphasizing the longer distance to be for alpha subunits rather than beta subunits. However, in this case the decrease in distances upon oxygenation was not significant for both alpha and beta subunits.

To verify whether the change in proximal-Fe^{2+} and distal-Fe^{2+} distance during oxygenation is driven by changes in histidine groups situations or by simple displacement of Fe^{2+} ion, we calculated proximal to distal histidine distances for each subunit and plotted them in Fig. 3.

Also, to understand the effect of oxygenation on proximal-distal distance, the percentages of change were calculated and summarized in Table 1. As shown, alpha subunits had 9-11% decrease in proximal-distal distance, while, beta subunits had only 2-3% decrease.

Moreover, the average distances between histidines of four subunits including proximal to proximal and distal to distal were calculated and presented in Table 2. The data in Table 2 provide an overview of hemoglobin tetrameric tertiary structure with proximal histidines placed in vicinity of central cavity and distal histidines in opposite direction as depicted in Scheme 1. Scheme 1 shows the spatial arrangement of hemoglobin subunits.

DISCUSSIONS

The average distance between proximal histidine and ferrous ions [Fe^{2+} - $N^{\varepsilon2}$ (*HisF* 8)] for hemoglobin subunits was calculated as an important parameter to compare the situation of proximal histidine in oxy (R state) and deoxy (T state). This parameter was deduced from 19 oxy and 41 deoxy coordinate structures and presented in Fig. 1. The figure indicates that distance is significantly shorter in beta subunits than alpha subunits *i.e.* 2.28 ± 0.02 against 2.41 ± 0.02. Also, it shows that Fe^{2+} - $N^{\varepsilon2}$ (*HisF* 8) distance
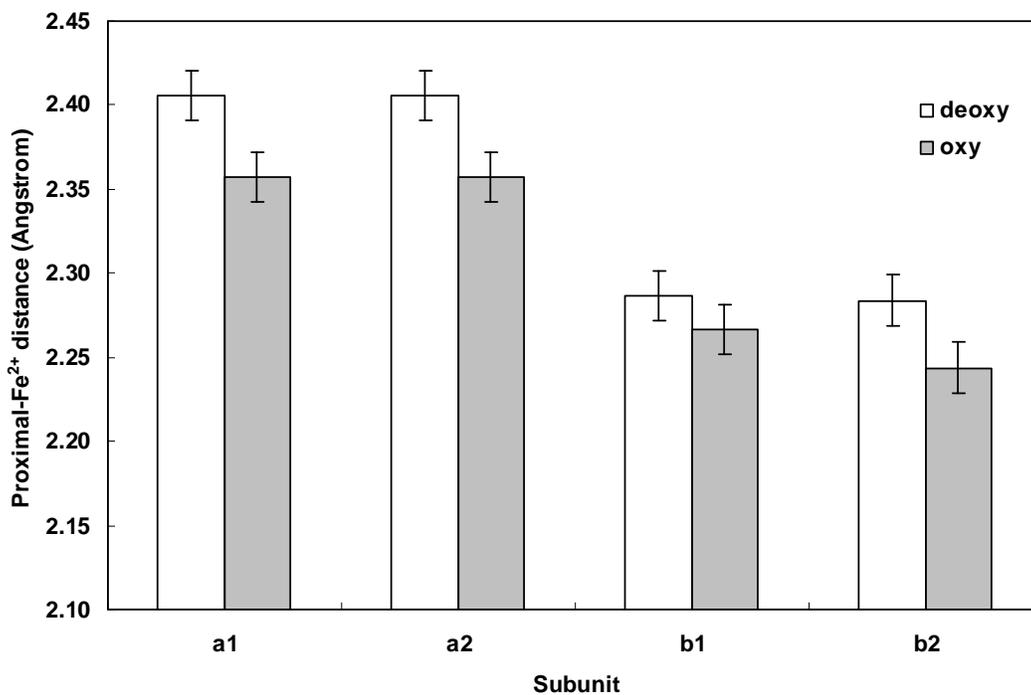
Fig. 1. Average distance (nm) between Fe^{2+} and its axial ligands of proximal histidine.
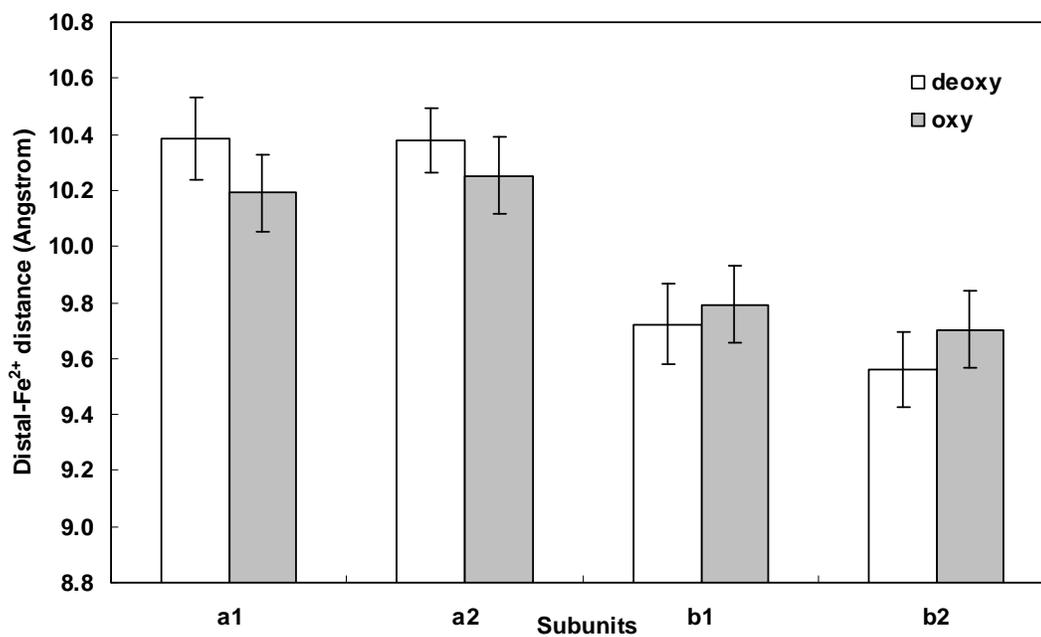
Fig. 2. Average distance (nm) between Fe^{2+} and its axial ligands of distal histidine.
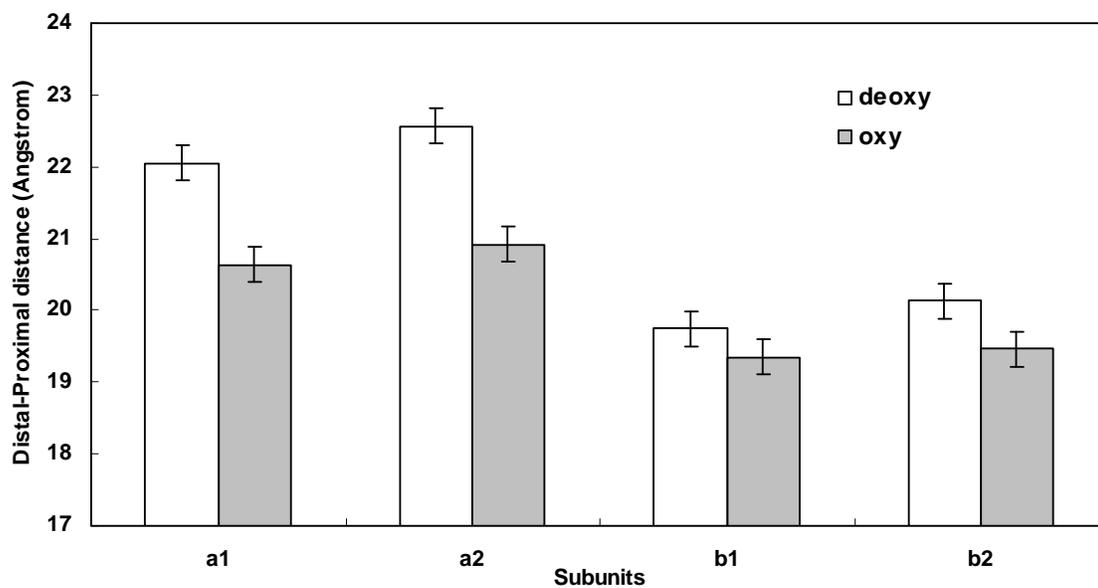
Fig. 3. Average distance (nm) of proximal and distal histidines in each subunit.

Table 1. Percentage Decrease in Proximal to Distal Histidine
in each Subunit upon Oxygenation

Subunits	Decrease in distance (%)
α_1	9
α_2	11
β_1	2
β_2	3

Table 2. Inter Subunits Distance (nm) of Proximal and Distal Histidines in Oxy
and Deoxy Conformation (Average ± Standard Error)

Subunits	Conformations	Proximal-Proximal	Distal-Distal
α_1-α_2	Deoxy	51.20 ± 1.02	82.83 ± 1.65
	Oxy	52.15 ± 1.04	88.31 ± 1.76
β_1-β_2	Deoxy	70.41 ± 1.40	104.69 ± 2.09
	Oxy	64.68 ± 1.29	103.39 ± 2.06
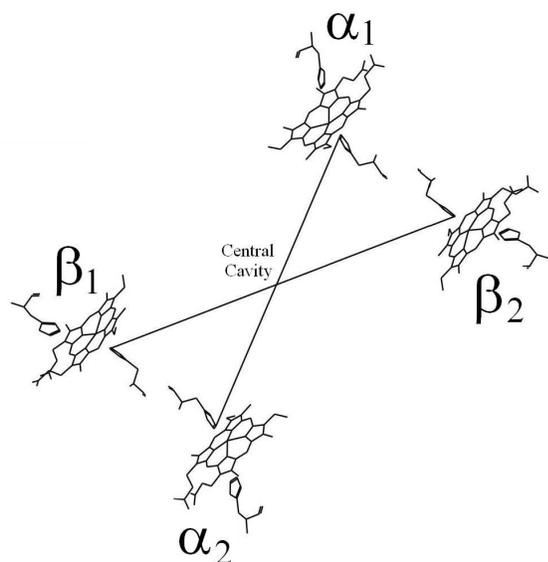
Scheme 1. Schematic representation of hemoglobin tetramer

expectedly decreases upon oxygenation of all subunits which causes proximal histidine to move down toward heme plane as described previously [Perutz, 1970]. We found that the decrease in Fe^{2+} - $N^{\varepsilon 2}$ (*HisF* 8) distances upon oxygenation was greater for alpha than beta subunits which manifest higher sensitivity of alpha subunits to oxygenation. Figure 2 shows the average distance between distal histidine and Fe^{2+} [Fe^{2+} - $N^{\varepsilon 2}$ (*HisE* 7)] for hemoglobin subunits in oxy and deoxy states. This distance was found to be about 4 fold larger than that of proximal histidine. This finding conveys that a longer covalent bond between distal histidine and Fe^{2+} Fe^{2+} - $N^{\varepsilon 2}$ (*HisE* 7) provides a weaker coordination bond to heme group which can be easily replaced by a molecular oxygen. This is the binding site by which hemoglobin carry oxygen to tissues. Figure 1 also shows that distal bond length is not affected by oxygenation of all subunits as reasonably expected.

Figure 3 illustrates the distance between proximal and distal histidine for each subunit. Since oxygen binding leads to downward movement of proximal histidine, the distance between proximal and distal histidine for each subunit is expected to decrease in oxygenated state. Figure 3 shows the distance between proximal and distal histidine decreased more obviously in alpha subunits than beta subunits. The relative decrease of distance of four subunits are

summarized in Table 1. As tabulated, the distance between proximal and distal histidine in α_1 and α_2 subunits indicates 9 and 11% decrease, respectively, while that in β_1 and β_2 subunits experiences only up to 2 and 3% decrease, respectively. Based on this finding, alpha subunits seem to be more responsive to oxygenation than beta subunits probably due to increased cooperativity. This finding is in good agreement with Fig. 1 and congruent with others' reports on higher cooperativity and affinity of alpha subunits [Rivetti *et al.* 1993, Bruno *et al.*, 2000, Maréchal *et al.*, 2006]. The data presented in Table 2 show that the distance between proximal histidine groups of subunits is about two third of that between distal histidine of each pair of subunits. This in turn means that hemoglobin tetramer arranged around each other in such a way that proximal histidines are hold toward each other directly in central cavity direction. Besides, the distance between proximal and distal histidine in alpha subunits is far lower than that in beta subunits. The fact that lead us to assume that tetrameric hemoglobin is not symmetrically structured. Finally, given the space filling model of hemoglobin tetramer (Scheme 1) and the data presented in Table 2, we found that the distance between proximal histidines of two alpha subunits increased from 51.20 ± 1.40 angstrom to 52.15 ± 1.04 angstrom upon oxygenation. This confirms farther movement of alpha subunits from each other.

In contrast, the distance between proximal histidines of two beta subunits decreased from 70.41 ± 1.40 nm to 64.68 ± 1.29 nm upon oxygenation which indicates beta subunits approaching each other. This finding is in agreement with Perutz's reports who showed that beta subunits move closer to each other upon oxygenation [Perutz *et al.*, 1964, Perutz, 1970]. This finding indicates that in oxy hemoglobin, subunits are distributed around central cavity in a more regular and symmetric pattern. While, in deoxy hemoglobin a deformed conformation represents more distant beta subunits than alpha subunits. This deformed conformation of deoxy hemoglobin may be accounted for the tens conformation [Bettati *et al.*, 1996, Cui & Karplus, 2008, Perrella & Russo, 2003]. Henceforth we postulate that the symmetric conformation of oxy hemoglobin could be considered as relaxed conformation with higher oxygen affinity.

CONCLUSIONS

Based on the findings derived from present study on crystal structures of human hemoglobin as well as from others' reports [Perrella & Russo, 2003, Schay *et al.*, 2006, Sahu *et al.*, 2007] we conclude that the tens conformations of hemoglobin has asymmetric conformation. Upon increase in oxygen pressure α_2 subunit of hemoglobin binds to molecular oxygen with higher affinity. Oxygen binding to Fe^{2+} ion pulls down proximal histidine. This movement in α_2 backbone seems to be reflected primarily in $\alpha_1\beta_2$ and $\alpha_2\beta_1$ interfaces causing partial change in their conformation from deoxy to oxy conformation. In next step, α_1 subunit of higher oxygen affinity and relaxed conformation accepts oxygen. Then, subunit β_2 which shows slightly more decrease in proximal to distal distance (3%) becomes the third subunit accepting oxygen. Finally β_1 subunit seems to be the final oxygen accepting subunit in our sequential model for hemoglobin.

ACKNOWLEDGEMENTS

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