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Structural studies on a low oxygen affinity hemoglobin from mammalian species: Sheep (*Ovis aries*)

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ABSTRACT

Hemoglobin (Hb) is in equilibrium between low affinity Tense (T) and high affinity Relaxed (R) states associated with its unliganded and liganded forms, respectively. Mammalian species can be classified into two groups on the basis of whether they express 'high' and 'low' oxygen affinity Hbs. Although Hbs from former group have been studied extensively, a limited number of structural studies have been performed for the low oxygen affinity Hbs. Here, the crystal structure of low oxygen affinity sheep methemoglobin (metHb) has been determined to 2.7 Å resolution. Even though sheep metHb adopts classical R state like quaternary structure, it shows localized quaternary and tertiary structural differences compared with other liganded Hb. The critical group of residues in the "joint region", shown as a major source of quaternary constraint on deoxyHb, formed unique interactions in the α1β2/α2β1 interfaces of sheep metHb structure. In addition, the constrained β subunits heme environment and the contraction of N-termini and A-helices of β subunits towards the molecular dyad are observed for sheep metHb structure. These observations provide the structural basis for a low oxygen affinity and blunt response to allosteric effector of sheep Hb.

1. Introduction

The allosteric mechanism of Hemoglobin (Hb) was primarily revealed by Perutz [1,2] and Baldwin and Chothia [3] when they determined the atomic structures of the classical T state (unliganded) and R state (liganded) forms of Hb, respectively embodied in the MWC model [4]. In the case of Hb, the oxygen affinity of the deoxyHb α2β2 tetramer is nearly 400-fold lower than that of the deoxy αβ dimers [5,6]. This clearly portrays the role of dimer–dimer interface quaternary constraints in deoxy tetramer for hampering the oxygen affinity. Structural analysis of the quaternary T and R states of Hb reveals similar contacts between α and β chains (α1β1), while in the other interfaces (α1α2/α1β2/α2β1) the contacts differ with respect to different states [7]. In α and β subunits, the C-terminal residues contribute to the salt bridges in stabilizing the structure in T state, which are absent in the liganded R state. These salt bridges are the major source of quaternary constraints in T state [1]. Besides the C-terminal salt bridges there are other ligation dependent dimer–dimer contacts present in the α1β2/α2β1 interface region of deoxyHb which could also serve as quaternary constraints. Recent investigations reveals that the critical group of residues mainly localized in the hinge region of α1β2/α2β1 interface significantly contribute to the quaternary constraints that result in the low oxygen affinity of the T state [8,9].

Mammalian Hb can be broadly classified into two groups: those with an intrinsically high oxygen affinity Hb, in which the oxygen affinity is lowered due to the presence of 2,3-diphosphoglycerate (DPG), an *in vivo* allosteric effector of mammalian Hb, and those with an intrinsically low oxygen affinity Hb, in which the action of DPG is limited or does not affect the oxygen affinity. Rodent, dog, pig, horse, camel, marsupial and most primates belong to the first category; cow, sheep, goat, deer, cat and the lemur, belong to the second [10]. Typically, Hbs with high intrinsic oxygen affinity have P_{50} values ranging from 4 to 6 mm-Hg, and those with low affinity possess a P_{50} value between 10 and 20 mm-Hg (measured in stripped Hb solutions in 0.05 M Bis-Tris, 0.1 M NaCl (pH 6.5–7.5)

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at 20–25 °C) [11]. While both high and low affinity Hbs have 141 amino acid residue α-chains, the high affinity and low affinity Hbs have 146 and 145 amino acid residue β-chains, respectively. Deletion or substitution of histidine in β-chain at the second (βH2) position has been suggested to be partly responsible for the ligand binding properties of low oxygen affinity Hbs [11]. βH2 is one among the several β-cleft residues that binds DPG, the principal allosteric effector of mammalian Hb [10,11]. The sheep Hb which is classified as a low oxygen affinity mammalian species possesses 10-fold lesser oxygen affinity than the human Hb [12]. The deletion of βH2 is expected to play a similar role as in low oxygen affinity species. Sheep Hb has formed part of this study because of its low oxygen affinity and ability to function without the aid of allosteric effector 2,3-diphosphoglycerate. In order to explore the structure function correlation of low oxygen affinity species, the crystal structure of sheep methoxy (met) hemoglobin was determined and reported here.

2. Materials and methods

2.1. Structure solution and refinement

Purification, crystallization and data collection statistics of sheep Hb has been published elsewhere [13]. Briefly, the sheep Hb crystallized in orthorhombic space group P 2_1 2_1 2_1 with unit cell dimensions $a = 60.23$, $b = 70.69$ and $c = 131.48$ Å. The crystal packing parameters revealed that one whole biological molecule (tetramer) in the asymmetric unit. The structure solution of sheep Hb was carried out by molecular replacement method using bovine carbonmonoxyHb (PDB ID: *1G08*) as a starting model, using the program *AMoRe* [14] implemented in the *CCP4* suite [15] programs. The rotational and translational function calculation using *AMoRe* yielded the best solution with a correlation coefficient of 66% and an R-factor of 42%. The structure refinement was carried out using *REFMAC* [16]. About 10% of reflections were used as the test set. The resultant model obtained from the structure solution was subjected to 20 cycles of rigid body refinement followed by few cycles of restrained refinement. At this stage the electron density map was calculated and manual rebuilding of the model was carried out using *COOT* [17] wherever necessary. This was followed by few rounds of restrained refinement using *REFMAC*. The water oxygens in the structure, including the heme coordinated water oxygens were identified by peaks greater than 3σ in Fo-Fc maps and 1σ in 2Fo-Fc maps. The structure was refined to a final R-factor of 20.1% and an R_{free} of 25.4%. The Ramachandran plot calculated for the final model with the program *PROCHECK* [18] shows that 91.5% of residues in the most favored region. The refinement parameters are listed in Table 1. The refined coordinates and structure factor have been deposited in the Protein Data Bank (PDB), with the accession number 2QU0. The ribbon representation of sheep metHb is shown in Fig. 1.

Table 1
Refinement statistics for crystal structure of sheep hemoglobin.

Resolution (Å)	30.0–2.7
No. of reflections	13,815
R_{work}/R_{free} (%)	20.1/25.4
R.m.s. deviations	
Bond length (Å)	0.013
Bond angle (°)	1.485
Mean *B*-values (Å^2)	40.26
Ramachandran plot	
Residues in most favorable regions (%)	91.5
Residues in additional allowed regions (%)	7.6
Residues in outlier region (%)	0.9

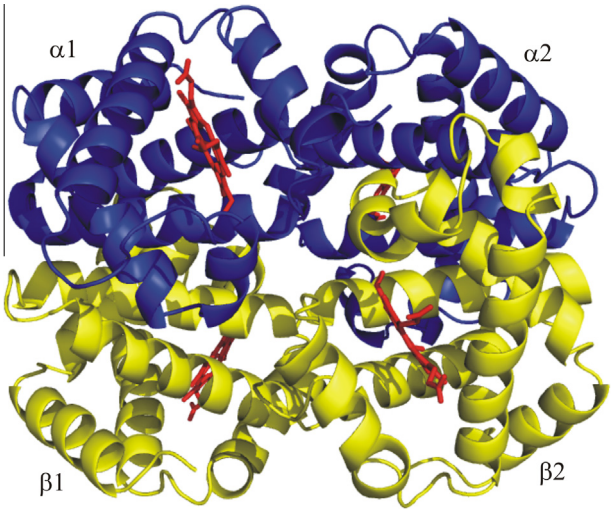

Fig. 1. Cartoon representation of sheep metHb with heme in each subunit.

3. Results and discussion

3.1. Sheep Hb polymorphism

Though there are different isoforms identified for sheep Hb, the primary structures are characterized only for Hb A, Hb B, Hb I and Hb K [19]. Initial studies revealed that two variants of sheep Hb namely Hb A and Hb B, consisting of identical α-chain, but consists of seven amino acid substitutions in the β-chain namely βS50N, βA58P, βV75M, βQ76K, βS120N, βE129D, βR144K (sheep Hb β-(A/B) subunit – Uniprot id: P02075), resulting in a Hb tetramer with different oxygen affinities [20]. The Hb B has lower oxygen affinity than the Hb A. Further studies on the sheep Hb revealed the existence of other isoforms due to different β-globin chains namely Hb E, Hb G, Hb I, Hb K and Hb H. The Hb G variant is shown to produce by mutation in Hb A type, while Hb I is characterized by a βG13S and the Hb K have βK66R and βK144R mutation of the β-globin of Hb B type, respectively [19]. In our sheep metHb structure, the electron density for Met75 is visible unequivocally in both Fo-Fc and 2Fo-Fc electron density maps indicating that it belongs to Hb B form. But under the given resolution limit one could not identify the other substitutions that are necessary to confirm the exact sequence of sheep Hb structure. Thus the above observations conclude that our sheep Hb structure belongs likely to the Hb B isoform.

3.2. Quaternary structural differences

For a measure of quaternary structure variation in sheep metHb, the α1β1 dimer reference frame is used to superimpose the sheep metHb structure with human T deoxyHb [21] (PDB ID: 2HHB) and human R oxyHb [22] (PDB ID: 1HHO). The C_α atoms (minus three residues at the terminal ends) are used for the superposition. The rmsd of the superimposed α1β1 dimer is reported in the top row of Table 2. The rmsd of the non-superimposed α2β2 dimers, and the rigid body rotation which relates the nonsuperimposed α2β2 dimers of the structures, were determined to illustrate quaternary structure differences between Hbs. These two quaternary indices are reported in the middle and bottom row of Table 2, respectively. The deviations among the α1β1 subunits provide a measure of the differences in the structure of the dimer, those in α2β2 largely depend upon the differences in the mutual orientation of the two dimers.

Table 2
Tertiary and quaternary structure differences of hemoglobins.

Hbs	Sheep met	Human oxy (R)	Human deoxy (T)
Sheep met	–	0.72	0.82
	–	0.97	4.68
	–	2.0	10.9
Human oxy (R)	0.72	–	0.83
	0.97	–	5.05
	2.0	–	12.8
Human deoxy (T)	0.82	0.83	–
	4.68	5.05	–
	10.9	12.8	–

For each pair of hemoglobin tetramers compared, the top entry corresponds to the rmsd of the superimposed α1β1 dimers. The middle entry corresponds to the rmsd between the nonsuperimposed α2β2. The bottom entry corresponds to the rigid body rotation relating the nonsuperimposed α2β2 dimers. The rmsds and rigid body rotation differences are in Å and degrees, respectively.

The superimposed rmsd value of α1β1 dimers of human T and R states of Hb is 0.83 Å, which is similar to the one between T and sheep metHb (0.82 Å). The rmsd between the R and sheep metHb is 0.72 Å. As expected, the results confirm that the internal structure of the α1β1 dimer is substantially conserved with respect to variation in ligand binding.

The rmsd of non-aligned α2β2 dimers between human T and R structure is 5.05 Å and that of T and sheep metHb is 4.68 Å. The large amount of quaternary structural changes occurs in α2β2 tetramer due to the ligand binding. The rmsd is 0.97 Å for R versus sheep metHb pair, which is comparable with their α1β1 deviation. The rigid body rotation angle amounts to the value of 12.8° between T and R states Hb and 10.9° between T and sheep metHb. The rotation angle between R and sheep metHb is 2.0°. The observed rmsd and rotation angle between T and sheep metHb is slightly lesser, but comparable with T–R transition. These analyses depict that the sheep metHb structure adopts a quaternary structure which is closer to human R oxyHb.

3.3. α1β2/α2β1 interface region

On transition from deoxy to liganded Hb, the significant structural changes are observed in the α1β2/α2β1 interface region [3]. The interactions between α1β2 and α2β1 are characterized by the diagnostic 'switch' and 'flexible joint' regions. FG corner and G helix residues (97–102) of the β subunit interact with C helix and CD corner residues (38–44) of the α subunit in the switch region, while in the joint region α subunit FG corner and G helix (91–97) residues interact with β subunit C helix and CD corner (35–43) residues in the α1β2/α2β1 interface region. Significant changes in the relative positions of the residues in the switch regions are noted during transition from T to R state, whereas the position of the residues in the joint region remains relatively unchanged. In T state, the switch region residue βH97 is nestled between αP44 and αT41 in both α1β2 and α2β1 interface regions. The transition to R state flips the residue βH97 to lie between αT38 and αT41. Similarly, βH97 positioned between αT38 and αT41 in α1β2 and α2β1 interfaces of sheep metHb substantiate its R state adoption.

In joint region, subtle but significant structural differences are observed in the α2β1 interface region of sheep metHb. Fig. 2A shows the stereoview of α2β1 joint region of sheep metHb with final 2Fo-Fc electron density map. To compare the α2β1 joint region interface of sheep metHb and human oxyHb, the structures were superimposed with the α1β1 reference frame. The superimposed α2β1 joint region of sheep metHb and human oxyHb is shown in Fig. 2B. In sheep metHb, the side chain conformation adopted by α2R92 differs significantly from the human oxyHb. Similarly, the CD corner residue β1E43 is shifted and oriented towards the interface region in sheep metHb when compared with human oxyHb. These structural changes in sheep metHb lead to unique interactions between α2R92 with β1E43 and α2R92 with β1P36 in α2β1 interface, but these interactions are found to be missing in the corresponding α1β2 interface region of sheep metHb, and also in human T and R states of Hb. The site directed mutagenesis studies [8] assure that the residues localized in the joint region of α1β2/α2β1 interface regions could play a prominent role in ligand affinity. Mutational studies emphasized that βW37 to Ala have larger impact on the ligand affinity with a 8-fold increase in CO binding to the deoxyHb tetramer. In deoxyHb, the side chain of βW37 is hydrogen bonded to αD94. The study also emphasize that the mutation of residues around the vicinity of the βW37 in the joint region could substantially affect the ligand affinity. The Ala mutant of joint region residues αR92 and βP36 increases the CO binding rate to deoxyHb by about 5 and 3-fold, respectively. In sheep metHb, αR92 and βP36 interacts through hydrogen bonding in the α2β1 joint region. The unique interactions formed by the critical residues in joint region impose additional quaternary constraints on sheep metHb and presumably lower its oxygen affinity.

In between the switch and joint region (termed as intermediate), the changes observed are mostly characterized by hydrogen bond patterns during the transition from T to R state. Comparing with the switch region, the changes in C_α–C_α distances between the residues in the intermediate region are relatively small during T–R transition. The intermediate region of sheep metHb resembles that of the R state hydrogen bonding pattern.

3.4. Heme structure

In sheep metHb, all four heme groups, as well as the heme coordinated water oxygens, are well defined by 2Fo-Fc electron density map. In the crystal structure of human oxyHb, all atoms of each heme group are planar [22], but in deoxyHb each heme iron deviates ~0.4 Å out-of-plane towards the proximal histidine [21]. In the present sheep metHb structure, Fe atoms lie out of plane from the mean porphyrin ring by 0.22 Å and 0.15 Å in the α- and β-heme. Table 3 presents the stereochemistry of the heme environment in sheep metHb, human metHb [23], human carbonmonoxyHb [24] and human oxyHb [22] structures. It appears that the non-covalent contacts between the Fe and the distal residues βH63 and βV67 at the two β hemes of sheep metHb are consistently shorter than the corresponding distances in the other liganded Hb structures. As pointed out by Perutz [1], the steric hindrance to ligand binding by these distal residues is dominant in β-subunit hemes and considered to be an additional contributing factor to the low oxygen affinity in T state. In contrast to the β heme group, no significant differences in these contacts are observed in the α-hemes. Fig. 3 illustrate the structural differences at the β subunit heme environment of the sheep metHb, human oxyHb [22] and human deoxyHb [21], respectively. The α1β1 reference frame was used in superposition of different structures.

3.5. The terminal regions

In the T state, the α subunit C-terminal residue, αR141 forms two ionic bonds with the opposite α subunit and contributes a hydrogen bond in α1β2 interface [1,25]. In β subunit, the C-terminal residue βH146 forms two salt bridges in T state [26]. Transition to the R state disrupts these salt bridges. The C-terminal salt bridge interactions imposes constraints on the T state structure and deletion and substitution mutations that cause loosening and weakening of any of the salt bridge interaction tend to increase the oxygen affinity in T state Hb by several fold [25–27]. The sheep metHb

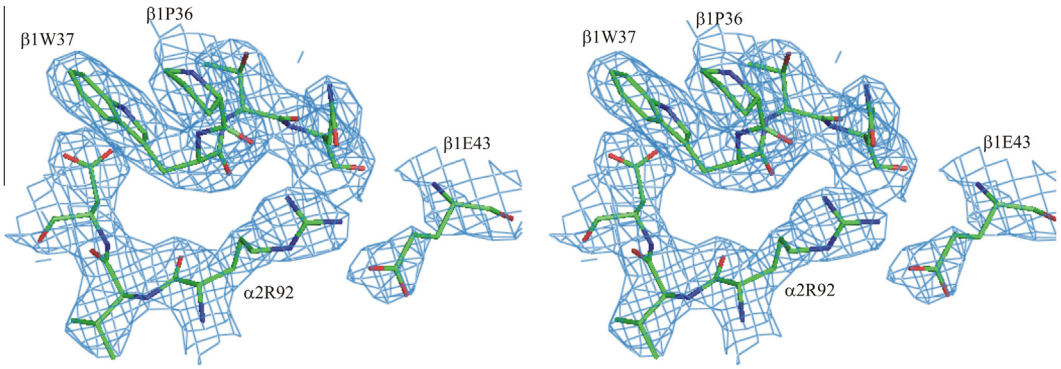

Fig. 2. (A) Stereoview of α2β1 joint region of sheep metHb in the 2Fo-Fc electron density map contoured at 1σ level.

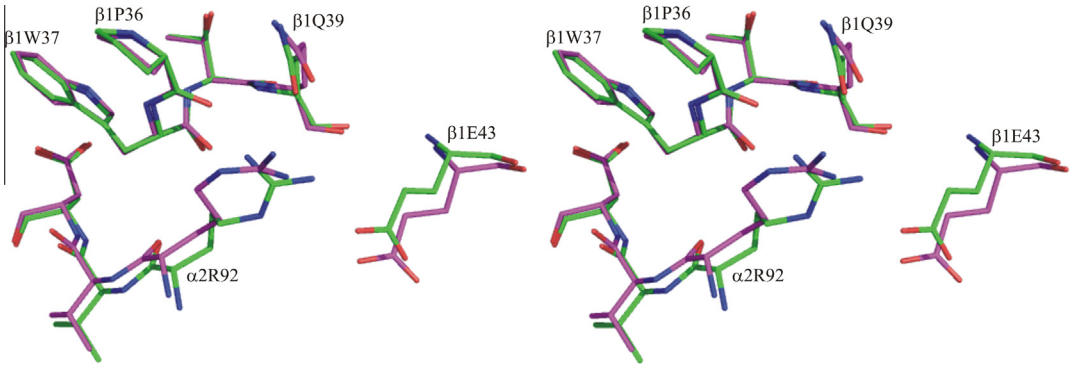

Fig. 2. (B) Stereoview of the α2β1 interface joint region of sheep metHb (green) superposed with human oxyHb (magenta). The structures were superposed with the α1β1 dimer reference frame. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Geometry of heme groups and its environment in hemoglobins.

Hbs	Subunit	Fe-His(E7)NE2 (Å)	Fe-Val(E11)CG2 (Å)	Fe-Phe(CD1)CZ (Å)	Fe-His(F8)NE2 (Å)	Fe-Plane distance* (Å)
Sheep Met	α1	4.39	4.99	5.72	2.01	0.22
	β1	4.11	4.46	5.38	1.96	0.15
	α2	4.33	5.20	5.66	2.00	0.22
	β2	4.21	4.66	5.32	2.02	0.18
Human met	α1	4.55	4.99	5.31	2.31	0.17
	β1	4.39	4.97	5.28	2.27	0.28
	α2	4.56	5.05	5.30	2.29	0.14
	β2	4.36	4.89	5.28	2.35	0.38
Human CO	α1	4.48	4.80	5.37	2.08	0.05
	β1	4.48	4.87	5.35	2.10	0.06
	α2	4.54	4.96	5.39	2.13	0.04
	β2	4.50	4.97	5.33	2.10	0.08
Human oxy	α1/α2	4.39	4.79	5.51	1.94	0.16
	β1/β2	4.19	4.66	5.66	2.07	0.06

* The plane is defined so as to pass through all heme atoms excluding the side chains and the Fe atom.

structure has clear densities for N and C-terminal residues in the α chain. The interactions formed by αR141 are found to be missing in sheep metHb alike in R state Hb. Besides the αR141, the adjacent residue αY140 could also have large impact on the ligand affinity, which αY140G variant react with CO 10-fold more rapidly than does human deoxyHb [8]. This residue has hydrogen bond interaction with αV98 from same chain in both T and R state Hbs. At the same time, this residue interacts with the joint region residues in α1β2/α2β1 interface. In deoxyHb, the α1Y140 side chain is in close van der Waals contact with β2W37 and the main chain is in weak contacts with the β2P36. The interaction with β2W37 is longer while interaction with β2P36 is missing in oxyHb. In sheep metHb, αY140 is having hydrogen bond interaction with the βP36 with a distance of 2.79 and 3.08 Å in the α1β2 and α2β1 interfaces, respectively. Specifically, the close van der Waals contact between the αY140 and βW37 is retained in the α2β1 interface region of sheep metHb. The observed interactions seems to have an impact on the mobility of the α subunit C-terminus of sheep metHb. The conformation of αR141 significantly differs from both the T and R state Hb.

For β-subunit C-terminal residues, the electron densities are not well defined (βY145, βH146) in sheep metHb. As a result, the interactions made by these residues are not clear in sheep metHb. The sheep metHb shows significant structural differences in N termini

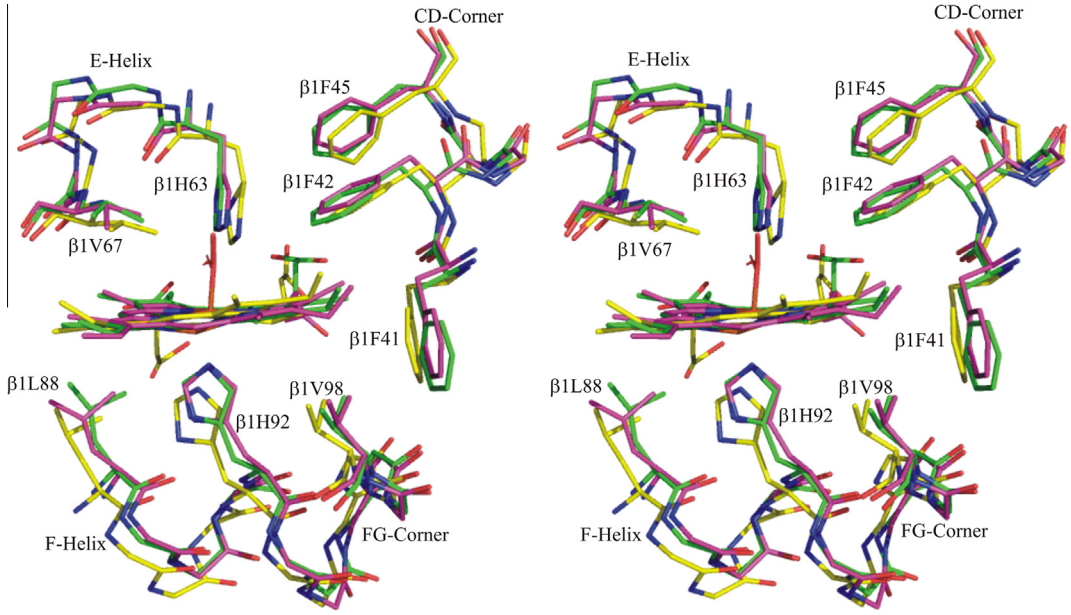

Fig. 3. Stereoview of the superimposed heme and its environment of sheep metHb (green), human oxyHb (magenta) and deoxyHb (yellow) for β1 subunit. The structures were superposed with the α1β1 dimer reference frame. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and A-helices of the β subunits. The N-termini and A-helices shift by 2.1 Å towards the molecular dyad in sheep metHb, relative to human oxyHb (Fig. 4). In human Hb, the same shift takes place during the binding of DPG [28]. In fact, the contraction of the N-terminus and A-helix in sheep metHb mimics the effect of DPG in Hb, which has been observed in bovine Hb and this movement is associated with its low oxygen affinity [29,30]. Thus from the above mentioned observations, the α subunit C-terminus region of sheep metHb maintains essential T state like quaternary structural features in the α1β2/α2β1 interfaces which may partly contribute to its low oxygen affinity. Together with the movement of the β subunits N-termini and A helices suggest that DPG cannot bind optimally, predicting the modest effect on sheep Hb affinity for oxygen.

Based on our structural analysis of sheep metHb presented here, we have substantiated the functional relevance for its low oxygen affinity and blunt response to DPG. The stabilized interface region due to unique interactions formed by the critical group of residues in the joint region seems to have a structural role on hampering the oxygen affinity of sheep Hb. Additionally, the observed constrained β subunits heme environments and contraction of the β subunits N-termini and A-helices toward the dyad exhibits the low oxygen affinity of sheep Hb.

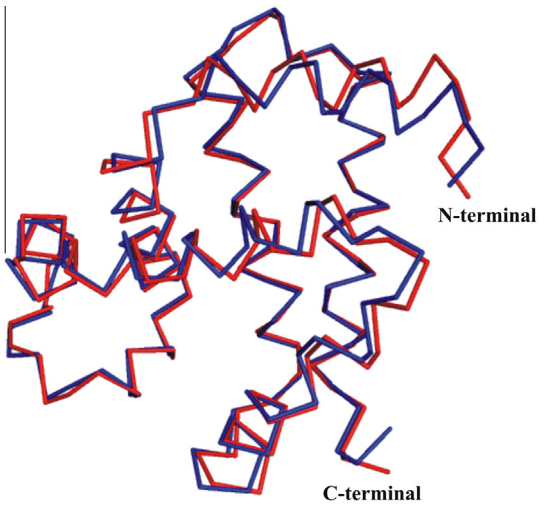

Fig. 4. Least-squares superposition of the β1 subunit between sheep metHb (blue) and human oxyHb (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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References

[1] M.F. Perutz, Stereochemistry of cooperative effects in haemoglobin, Nature 228 (1970) 726–739.
[2] M.F. Perutz, Nature of haem–haem interaction, Nature 237 (1972) 495–499.
[3] J. Baldwin, C. Chothia, Haemoglobin: the structural changes related to ligand binding and its allosteric mechanism, J. Mol. Biol. 129 (1979) 175–220.
[4] J. Monod, J. Wyman, J.P. Changeux, On the nature of allosteric transitions: a plausible model, J. Mol. Biol. 12 (1965) 88–118.
[5] F.C. Mills, M.L. Johnson, G.K. Ackers, Oxygenation-linked subunit interactions in human hemoglobin: experimental studies on the concentration dependence of oxygenation curves, Biochemistry 15 (1976) 5350–5362.
[6] G.K. Ackers, J.M. Holt, Y. Huang, Y. Grinkova, A.L. Klinger, I. Denisov, Confirmation of a unique intra-dimer cooperativity in the human hemoglobin alpha(1)beta(1)half-oxygenated intermediate supports the symmetry rule model of allosteric regulation, Proteins 4 (2000) 23–43.
[7] C. Chothia, S. Wodak, J. Janin, Role of subunit interfaces in the allosteric mechanism of hemoglobin, Proc. Natl. Acad. Sci. U.S.A. 73 (1976) 3793–3797.
[8] R.W. Noble, H.L. Hui, L.D. Kwiatkowski, P. Paily, A. DeYoung, A. Wierzba, J.E. Colby, S. Bruno, A. Mozzarelli, Mutational effects at the subunit interfaces of human hemoglobin: evidence for a unique sensitivity of the T quaternary state to changes in the hinge region of the α1β2 interface, Biochemistry 40 (2001) 12357–12368.
[9] J.S. Kavanaugh, P.H. Rogers, A. Arnone, Crystallographic evidence for a new ensemble of ligand-induced allosteric transitions in hemoglobin: the T-to-T-high quaternary transitions, Biochemistry 44 (2005) 6101–6121.
[10] H.F. Bunn, Differences in the interaction of 2,3-diphosphoglycerate with certain mammalian hemoglobins, Science 172 (1971) 1049–1050.
[11] M.F. Perutz, K. Imai, Regulation of oxygen affinity of mammalian haemoglobins, J. Mol. Biol. 136 (1980) 183–191.

[12] R. Benesch, R.E. Benesch, C.I. Yu, Reciprocal binding of oxygen and diphosphoglycerate by human hemoglobin, Proc. Natl. Acad. Sci. U.S.A. 59 (1968) 526–532.
[13] K. Neelagandan, Pon. SathyaMoorthy, M. Balasubramanian, M.N. Ponnuswamy, Crystallization of sheep (*Ovis aries*) and goat (*Capra hircus*) haemoglobins under unbuffered low-salt conditions, Acta Crystallogr. F Struct. Biol. Cryst. Commun. 63 (2007) 887–889.
[14] J. Navaza, *AMoRe*: an automated package for molecular replacement, Acta Crystallogr. A50 (1994) 157–163.
[15] Collaborative Computational Project, Number 4, The *CCP*4 suite: programs for protein crystallography, Acta Crystallogr. D Biol. Crystallogr. 50 (1994) 760–763.
[16] G.N. Murshudov, A.A. Vagin, E.J. Dodson, Refinement of macromolecular structures by the maximum-likelihood method, Acta Crystallogr. D Biol. Crystallogr. 53 (1997) 240–255.
[17] P. Emsley, K. Cowtan, Coot: model-building tools for molecular graphics, Acta Crystallogr. D Biol. Crystallogr. 60 (2004) 2126–2132.
[18] R.A. Laskowski, M.W. MacAuthur, D.S. Moss, J.M. Thornton, *PROCHECK*: a program to check the stereochemical quality of protein structures, J. Appl. Crystallogr. 26 (1993) 283–291.
[19] L. Manca, M. Corda, M. Pellegrini, A. Fais, E. Hadjisterkotis, A. Nàhlik, A. Basile, P. Ferranti, B. Masala, Structure and function of sheep hemoglobin chios: a novel allele at the HBBB locus with two Lys → Arg substitutions at positions β66(E10) and β144(HC1), Comp. Biochem. Physiol. 2D (2007) 84–90.
[20] F.H. Bunn, B.G. Forget, Animal hemoglobins, in: F.H. Bunn, B.G. Forget (Eds.), Hemoglobin: Molecular, Genetic and Clinical Aspects, W.B. Saunders, Philadelphia, 1986, pp. 126–167.
[21] G. Fermi, M.F. Perutz, B. Shaanan, R. Fourme, The crystal structure of human deoxyhaemoglobin at 1.74 Å resolution, J. Mol. Biol. 175 (1984) 159–174.
[22] B. Shaanan, Structure of human oxyhaemoglobin at 2.1 Å resolution, J. Mol. Biol. 171 (1983) 31–59.
[23] B.K. Biswal, M. Vijayan, Structure of human methaemoglobin: the variation of a theme, Curr. Sci. India 81 (2001) 1100–1105.
[24] M.M. Silva, P.H. Rogers, A. Arnone, A third quaternary structure of human hemoglobin A at 1.7-Å resolution, J. Biol. Chem. 267 (1992) 17248–17256.
[25] J.S. Kavanaugh, D.R. Chafin, A. Arnone, A. Mozzarelli, C. Rivetti, G.L. Rossi, L.D. Kwiatkowski, R.W. Noble, Structure and oxygen affinity of crystalline desArg141α human hemoglobin A in the T state, J. Mol. Biol. 248 (1995) 136–150.
[26] S. Bettati, L.D. Kwiatkowski, J.S. Kavanaugh, A. Mozzarelli, A. Arnone, G.L. Rossi, R.W. Noble, Structure and oxygen affinity of crystalline des-His-146β human hemoglobin in the T state, J. Biol. Chem. 272 (1997) 33077–33084.
[27] R. Liddington, Z. Derewenda, G. Dodson, D. Harris, Structure of the liganded T state of haemoglobin identifies the origin of cooperative oxygen binding, Nature 331 (1988) 725–728.
[28] A. Arnone, X-ray diffraction study of binding of 2,3-diphosphoglycerate to human deoxyhaemoglobin, Nature 237 (1972) 146–149.
[29] M.F. Perutz, G. Fermi, C. Poyart, J. Pagnier, J. Kister, A novel allosteric mechanism in haemoglobin. Structure of bovine deoxyhaemoglobin, absence of specific chloride-binding sites and origin of the chloride-linked Bohr effect in bovine and human haemoglobin, J. Mol. Biol. 233 (1993) 536–545.
[30] M.K. Safo, D.J. Abraham, The X-ray structure determination of bovine carbonmonoxy hemoglobin at 2.1 Å resolution and its relationship to the quaternary structures of other hemoglobin crystal forms, Protein Sci. 10 (2001) 1091–1099.