Structural and functional diversity of haemoglobin molecule properties amongst different classes and species of animals

Mahima Sanyal, Paurnima Patil, Ekta Jaiswal, Aparna Deshmukh

Department of Biotechnology, Thakur College of Science and Commerce, Kandivali, Mumbai 400101, Maharashtra, India, Email: jaiswal.ekta@outlook.com

ABSTRACT

Haemoglobin is one of the extensively studied molecules in the vertebrates. The ancestral gene for haemoglobin (Hb) is ancient and has evolved from bacteria to humans. The functional properties of haemoglobin and its modulation by allosteric effectors have often been interpreted as adaptations to environment and physiological demand of species and may serve additional functions besides transport of oxygen between tissues. Haemoglobin of different species show varying oxygen carrying capacity. In reptiles, the cardiovascular system show low fluid resistance due to widely spaced capillaries as compared to mammals and haemoglobin shows reduced oxygen transport ability. In birds, haemoglobin is highly similar to mammalian haemoglobin, both structurally and in its amino acid sequence. The unusually high oxygen affinity of Hb of avian species represents an adaptation to high altitudes. In fishes, the haemoglobin concentration varies according to environmental conditions. Fishes thriving in polar habitats have evolved reduction of haemoglobin concentration and multiplicity possibly as a consequence of temperature stability and other parameters. This review presents comparative analysis of haemoglobin to understand protein adaptations with respect to temperature and variation in species.

Keywords: haemoglobin, oxygen affinity, amino acid substitutions, conformational shifts

INTRODUCTION

Haemoglobin is the most abundant and vital protein amongst the array of haemproteins. It is the most intensively studied protein whose behavior can be described in terms of basic stereo chemical reactions. Human adult haemoglobin (HbA) is a tetrameric molecule which is made up of four subunits and consists of two alpha- and two beta-polypeptide chains, each containing one haem group. It is the precise amino acid sequence of the alpha and beta chains that determines the folding of each chain and it is the interaction between the subunits that refines the oxygen binding properties. A single molecule concerned with the crucial role of oxygen transport, is able to equip itself to perform activities in unrelenting conditions faced by organisms such as the Emperor Penguins, creates base to exploit its structural features in other organisms, for making the molecule adept for therapeutic purposes, should a similar clinical abnormality manifest.

Haemoglobins are usually thought of as the major proteins in erythrocytes circulating in the blood of vertebrates, carrying the oxygen inhaled by the lungs to the respiring tissues in the body. Haemoglobin has been found in almost all kingdoms of organisms. This suggests that the ancestral gene for hemoglobin is ancient, and that haemoglobins can serve additional functions

Invited Review, Acta Biologica Indica 2013, 2(2):381-387

pISSN 2319-1244, eISSN 2279-0160

besides transport of oxygen between tissues, ranging from intracellular oxygen transport to catalysis of redox reactions [1]. These different functions of the haemoglobins illustrate the acquisition of new roles by a pre-existing structural gene, which requires changes not only in the coding regions but also in the regulatory elements of the genes. Crucial positions determine the binding properties of hemoglobin, such as that seen in autooxidation properties of fish haemoglobin that could be narrowed down as a point of approach in modulating the properties of a molecule. At an early stage in evolution, worm-like animals had large, polymeric aggregations of Hb subunits circulating through primitive circulatory systems and some possessed monomeric Hb in blood cells functioning as an oxygen store. Polymerization, a feature largely seen in reptilian hemoglobin which bears close resemblance to embryonic haemoglobins, is now being procreated as a potential RBC substitute.

The predominant structural state of the molecule greatly depicts its adjustment for a more suited role in a given microenvironment. Oxygen binding is associated with small changes in the tertiary structure of segments near the haem and a large shift in the quaternary structure from the T (tensed) to the R (relaxed) state, as one dimer rotates relative to the other. Pertaining to this, structural characteristics present in the altitude adapted Guinea Pig hemoglobin, renders it to be present at a higher oxygen affinity conformational state. The fine structural differences that come along with an induced conformational change and presence of crucial amino acid positions, adapt to the asking conditions. The circulating vertebrate red blood cell provides an environment allowing haem units to interact among themselves and with various organic phosphates to allow a responsive and highly regulated system of gas transport.

ALPHA-D GLOBIN, OXYGEN AFFINITY AS STRUCTURAL INFLUENCES IN REPTILES AND BIRD Hb BASED ON ENVIRONMENTAL CONDITIONS

Reptiles are a diverse assemblage of animals which represent the evolutionary transition to fully terrestrial tetrapod life. Amniota consisting of reptiles, birds and mammal, in general have two or more hemoglobin components. These are expressed according to the demands of different physiological conditions. Among them, hemoglobin D (Hb D) was first found in birds then in reptiles as a minor component of the embryonic and adult definitive erythrocytes [2]. Knapp et al. brought out that the higher stability of avian Hb compared to that of mammalian Hb was due to the difference in the influence of HbD and might be one such adaptation of insufficient oxygen supply as observed in the embryonic stages or extreme hypoxic and even anoxic conditions. Tolerance to hypoxia in a number of birds, such as the high soaring vultures and migratory ducks and geese [3] appear to stem from as a result of functional differentiation of HbA and HbD isoforms. AlphaD globin has raised interests in relation to the biochemical adaptations, that was shown to bear resemblance with the embryonic hemogloboin primary structure coupled with the evolutionary distribution of this molecule that has restricted it to Aves and Reptilia. The structural features of the hemoglobin component HbA (alpha A2 beta2) and HbD (alpha D2 beta 2) as found in the RBCs of adult Western Painted Turtles that adapt to extreme hypoxic conditions during hibernation are accompanied by high oxygen affinity [4].

OXYGEN AFFINITY, EFFECTORS AND pH EFFECT ON THE STRUCTURAL ASPECTS OF REPTILES AND BIRDSHEMOGLOBIN

P50 values of turtle Hb, irrespective of the presence or absence of allosteric effectors, are significantly higher than those of human Hemoglobin. This can be illustrated by the findings that the effect of chloride is three times lower in turtle than in human or bovine Hemoglobin (Hb). The turtle Hb displays a Bohr Effect characterized by a substantial shift of the mid-point of the transition

towards acidic pH values. The Bohr Effect of turtle Hb which is approximately 50% of that of HbA is completely abolished in the absence of chloride ions, i.e., the pH dependence is completely abolished in the absence of the ion. To bolster this, Blood pH data of emperor penguins during dives have demonstrated that the blood does not become very acidotic during the dive, indicating that the effects of the Bohr shift may not be particularly relevant during diving in this species [5]. The lowered oxygen affinity of turtle Hb can be attributed to a hydrophobic moiety causing interruptions in essential bindings otherwise in human hemoglobin. In bovine Hb the alpha helix and the N terminal residues are shifted closer to the main body of the beta chains, an effect similarly seen on DPG binding to hemoglobin binding, rendering the molecule to have low oxygen affinity [6].

EFFECT OF SUBUNIT INTERACTIONS AND STABILITY IN BIRDS, REPTILES AND MAMMALS

Stability of a variant subunit is an important factor that influences the amount of variant hemoglobin in the red cell. Coupling with this factor, are assisting influences such as normal rates of transcription and translation of these variant subunits [7]. Distribution of haemoglobins in the red cell is affected by small differences in the rates of combination of variant subunits with normal partner subunits. This can be illustrated by alpha thalassemia, characterized by limiting amount of alpha chains, decreasing the proportion of positively charged variant. Normal and beta subunits compete for alpha subunits and represent various stages of haemoglobin development in erythroblasts of heterozygotes. Alpha haemoglobin subunits when at their isoelectric point are unaffected by the differences in charge on the beta subunit on the rate of alpha dimer formation [8]. The Delta globin subunit is considerably more positively charged than is the beta subunit. Substitution of cysteine to threonine at 112^{th} position facilitates the relatively slower rate of alpha gamma dissociation at high pH, as compared to that of alpha beta. Clear evidence shows that Hbs containing alpha-subunits have much stronger interactions with their partner subunits than do those containing f-subunits [7]. Fetal Hb is the least dissociated. Presence of a large tetramer interface due to the presence of an alpha D subunit causes the distal histidine to be pushed further into the haeme pocket thereby hindering oxygen binding and reducing oxygen affinity [9].

Presence of Hb D component as is noted in Hb from several birds induces a lower oxygen affinity and that formation of a large tetramer-tetramer interface in chicken Hb D mediates a shift in the helix of alpha beta subunits in such a way that the distal histidine is pushed further into the haem pocket, as it was also observed in lamprey deoxy Hb [10]. Since the resulting position of the distal histidine hinders the oxygen binding, the oxygen affinity is reduced. Significant differences in alpha1beta1 and alpha2beta2 packing sites have been elucidated on comparison of human Hb with logger sea turtle [1]. The absolute oxygen affinities in vertebrate haemoglobins are subject to modulations that help the molecule to meet the physiological demands. These modulations, governed by temperature and heterotopic ligands, are reflective of thermodynamic linkages that the Hb molecule makes with oxygen and allosteric effectors. Ligand binding induces quaternary conformational change which remains largely unchanged at the alpha1beta1 contact site. Substitutions such as that found in *C. caretta* beta chain Beta108(G10) Asn > Ser perturb the oxygen affinities and is considered a crucial determinant as it has been found to be common to other diving animals such as the emperor penguins.

POLYMERIZATION: A PROPERTY TO EXTRAPOLATE

Hydrophobic interactions affect polymerization of biomolecules [11]. Hydration affects the empirical conformational energy values [12,13]. The free energy of hydration is composed of

additive contributions of various functional groups of proteins. The hydration of each group is assumed to be proportional to the accessible surface area of the group [14]. The energy of unfolding is considered as a measure of protein stability [14,15]. Hb D induces turbidity and polymerization in the avian Hb. Various species of turtles have also been examined to have haemoglobins with heavy components. A very small quantity of a 12-13S component was observed in the hemolysates from both the snapturtle and the bullfrog, when few species of turtle were examined to have haemoglobins with heavy components. Haemoglobins from different species of turtle vary considerably in pH and concentration dependence of oxygen equilibria.

Polymerization of 4S Hb molecules to 7S molecules occurs in turtle and bull hemoglobins and that this polymerization is triggered at the time of hemolysis. Sulfhydryl groups might be involved in forming inter-chain disulphide bridges. Hemoglobin based oxygen carriers HBOC are being developed as RBC substitutes. However vasoconstriction and hypertension still remain significant side effects. The major chemical reaction in the polymerization of hHb with glutaraldehyde involves Michael addition between alpha beta unsaturated oligomeric aldehydes and primary amine groups on lysine residues that are present on the surface of hHb. Therefore, polymerization of hHb is based on stable C-N bonds that will not hydrolyze in solution [16].

STRUCTURAL CHANGES IN BIRD Hb IN RESPONSE TO EXTREME CONDITIONS

The emperor penguin (*Aptenodytes forsteri*), the consummate avian diver, thrives in the extreme Antarctic underwater environment, diving to depths greater than 500 m [17]. It has been hypothesized that this underlying tolerance, well below the limits of many birds and mammals, may necessitate biochemical and molecular adaptations including a shift in the emperor penguin Oxygen-hemoglobin (Hb) dissociation curve, relative to other birds. In general, the hemoglobin of birds has lower Oxygen affinity than that of mammals [18]. This may reflect a shift toward favoring Oxygen unloading to the tissues, as the avian respiratory system is inherently more efficient at Oxygen uptake [19,20].

Meir et al. found that as compared to human Hb, there are changes in six alpha chain and five beta-chain $\alpha 1\beta 1$ contact sites, and one alpha-chain $\alpha 1\beta 2$ contact site. It is possible that one of these modifications accounts for the emperor penguin's increased Hb-Oxygen Affinity. The Oxygen-Hb dissociation curve of the emperor penguin is left-shifted relative to most birds, similar to that of other penguin species and the high-flying bar-headed goose [22,23]. A small Bohr effect is a potential disadvantage in terms of unloading Oxygen to the tissues in a diving animal, especially in those with relatively high Hb-Oxygen affinities [24]. Blood pH data during dives have also demonstrated that emperor penguin blood does not become very acidotic during the dive, indicating that the effects of the Bohr shift may not be particularly relevant during diving in this species[5,25]. Left-shifted hemoglobin (higher Hb-Oxygen affinity) is advantageous for the diving emperor penguin, as it implies that more Oxygen is available at any given PO2. The increased Oxygen affinity of emperor penguin Hb allows for continued extraction of this Oxygen from the respiratory Oxygen store for use during diving.

STRUCTURAL CHANGES FOUND IN FISH SPECIES AS AN ADAPTATION TO BRING ABOUT EFFICIENT OXYGEN USAGE

Temperature and ambient oxygen availability affects the hemoglobin Oxygen binding affinity of poikilothermic animals, influencing its maintenance and activity. Optimizing oxygen transport to suit to various temperature dependent requirements calls for functionally different hemoglobins

isoforms [26]. The nucleated red blood cells of fishes are generally characterized by multiple haemoglobin components, which are functionally different and appear adapted for oxygen extraction in habitats that may vary in oxygen, temperature and carbon dioxide [27,28]. Assessment of a strong association between the two haplotypes Met55-Lys62 and Val55-Ala62 showed that replacement of the Met55 with the smaller Val residue increases the alpha1 beta1 subunit distance, probably reducing the dimer stability [8]. Correspondingly acting, is the effect of Lys62BetaAla polymorphism. Water, stabilized by polar residues hinders ligand access to distal pocket residues. A stronger water interaction occurs with Lys as compared to Ala, as studied by determining the binding energy of water probe with distal heme pocket. Thereby, reinforcing the role of distal pocket residues in the oxygen binding regulation [29]. These crucial positions are mutated in the hemoglobin of the hypoxia tolerant Andean goose (Leu55beta → Ser) thus surfacing the importance of these residues to adapt optimally to the environmental conditions [26,30].

Autooxidation rates of fish hemoglobin is based on a pH dependent scale against human hemoglobin: at pH 5.7 and 6.3, Pouch and Trout Hbs have 30 to 80 fold higher autooxidation rates, wherein at pH 8 the rate falls to about two times as rapid. The sequence identity for Trout IV and Perch Hb alpha subunits as compared with bovine Hb, amounts to: 54 and 55 % respectively and that with beta subunits, 47% identity is seen. These differences, predominantly existing outside the heme pocket, present as significant residual changes in the heme pocket of fish alpha and beta subunits as compared to bovine Hb subunits. This increase was attributed to hindrance by the δ methyl group of Ile (E11), which is located much closer to the heme iron atom of the heme moiety than the γ methyl group of the shorter Val (E11) side chain, as a consequence the bound ligand faces strong steric clashes creating disorders in the conformation of the E11 side chain and the geomtery of the bound ligand. In addition, ThreE10 present in both subunits of both fish Hbs, in contrast to the LysE10 in bovine Hb, are too short to hydrogen bond, either directly or indirectly through water molecule, to the 7-propionate. As a result there are no favorable electrostatic interactions of ThrE10 with the heme group, increasing the hemin release rate. The extent of accessibility to solvent of the internal edges of the heme also Influence autooxidation and hemin loss by facilitating unfolding of the helices. Gly E14 brings about this effect in TroutIV Hb [26].

FEATURES THAT STAND OUT IN THE MAMMALIAN SPECIES

Life at high altitudes is markedly characterized by low oxygen availability, which challenges aerobic metabolism. Vertebrates show a remarkable ability to adapt to life under these conditions by developing several strategies both on the organismic and molecular level to alleviate the effects of low oxygen availability. The hemoglobin of new world camelids such as llama and guanaco, which live in altitudes up to 5000 m, has a partly degenerated binding site for the allosteric effector 2,3-bisphosphoglycerate caused by a His2 → Asn mutation on the Beta-subunit. In contrast, hemoglobins of guinea pig, an organism adapted to high altitude, do not show this change and have normal sensitivity towards 2,3-bisphosphoglycerate [31].

The R2 state in guinea pig hemoglobin is better stabilized and is found to be present in the physiologically relaxed state than that in human Hb where R state is found to be in the physiologically relaxed conditions. In addition, destabilization of T state contributes to the higher oxygen affinity of guinea pig Hb than that of human Hb [32]. Baldwin and Chothia described the importance in switch region conformation changes due to the amino acids in achieving the higher oxygen affinity, unlike in Human Hb where the switch region is stabilized by a salt bridge between Glu30 and His50. These residues are absent in guinea pig. The T state His97 of the Beta-2 subunit is positioned between Thre41 and Pro44 of the alpha1 subunit. In Guinea pig, the Pro44 is changed to His44. Positioning of His97 just opposite His44 stearically hinders the conformation, resulting in a less stable T state and a higher Oxygen affinity. Transition to R state, pushes His97 along one turn

of C helix - alpha helix and positions it between Thr38 and Thr42. In the R2 state the His is positioned exactly opposite the Thr38. These positional shifts push the equilibrium towards the more relaxed states. Perhaps the most striking feature that demonstrates the R ↔ R2 intermediate nature is the location of the beta2 His-97 side chain [34]. These structural characteristics present in the Guinea Pig hemoglobin, puts the R2 state at higher stability and renders the T state less stable, attributes the molecule to have a greater oxygen affinity.

CONCLUSION

The functional differences in the hemoglobin variants like alpha D globin which bears resemblance to the human embryonic hemoglobin, have shown to adapt to the hypoxic conditions. The presence of various effectors and its utility as an influence to the hemoglobin molecule properties varies with the kind of environment that is posed to the organism. The kind of interaction of the subunits modifies between and across species, fine tuning the need to optimize its potential to the environmental conditions. Polymerization of the hemoglobin molecule, a property seen in reptilian species, as a mode to recover from hemolysis, has now been realized as a potential RBC substitute for storage. Such numerous differences in the properties of the same molecule serve as a tool to optimize it for correction of any clinical abnormality that could possibly manifest.

REFERENCES

[1] Hardison R. J Exp Biol 1998, 201:1099-1117.
[2] Brown JL, Ingram H. J Biol Chem 1974, 249:3960-3972.
[3] Hiebl L, Weber RE, Schneeganss D, et al. J Biol Chem 1988, 369(4):217-232.
[4] Abbasi A, Braunitzer G. J Biol Chem 1985, 366:699-704.
[5] Ponganis, PJ, Kooyman GL. Physiol Zool 1993, 66:732-749.
[6] Petruzzelli R, Aureli G, Lania A, et al. Biochem J. 1996, 316:959-965.
[7] Manning LR, Russell JE, Padovan JC, et al. Protein Sci. 2007, 16:1641-1658.
[8] Bunn HF. Blood 1987, 69:1-6.
[9] Aranda R, Cai H, Worley CE, et al. Proteins 2009, 75(1):217-230.
[10] Ajloo D, Moosavi-Movahedi AA, Sadeghi M, Gharibi H. Acta Biochemica Polonica 2002, 49(2):459-470.
[11] Urry DW, Gowda DC, Parker TM. Biopolymers 1992, 32:1243-1250.
[12] Adachi K, Kim J, Travitz R, et al. Biol Chem 1987, 262:12920-12925.
[13] Yohe ME, Sheffield KM, Mukerji I. Biophys J 2000, 78:3218-3226.
[14] Ooi T, Oobatake M, Nemethy G, Scherega HA. Proc Natl Acad Sci. 1987, 84:3086-3090.
[15] Jiang X, Kowalski J, Kelly JW. Protein Sci. 2001, 10(7):1454-1465.
[16] Zhang N, Jia Y, Chen G, et al. Tissue Engineering Part A 2011, 17(7-8):927-940.
[17] Wlenecke B, Robertson G, Kirkwood R, Lawton K. Polar Biol. 2007, 30:133-142.
[18] Jessen TH, Weber RE, Fermi G, et al. Proc Natl Acad Sci. 1991, 88:6519-6522.
[19] Piiper J, Scheld P. Respir Physiol. 1975, 23:592-594.
[20] Powell FL, Wagner PD. Respir Physiol. 1982, 48:233-241
[21] Meir JU, Ponganis PJ. J Exp Biol 2009, 212:3330-3338.
[22] Black CP. Resp Physiol 1980, 39:217-239.
[23] Milsom WK, Johansen K, Millard RW. Condor 1973, 75:472-474.
[24] Lenfant C. In The Biology of Marine Animals, Anderson HT (ed.), Academic Press, New York, 1969, 95-116.
[25] Petschow D, Wurdinger I, Baumann R, et al. Resp Physiol 1977, 42:139-143.
[26] Andersen O, Wetten OF, De Rosa MC, et al. Proceedings of the Royal Society 2009, 276:833-841.
[27] Perez J, Rylander K, Nirchio M. Rev Fish Biol Fish 1995, 5:304-319.
[28] Berebrink M. Science 2007, 307:1752-1757.
[29] Springer B. Chem Rev 1994, 94:699-714.

[30] Kachalova G. Science 1999, 284:473-476.
[31] Bunn H. Science 1971, 172:1049-1050.
[32] Palret B, Jaenicke E. PLoS One 2010, 5(8):e12389.
[33] Baldwin J, Chothia C. J Mol Biol 1979, 129:175-220.
[34] Schumacher M. Proc Natl Acad Sci 1997, 94:7841-7844.