Biophysical Chemistry, 37 (1990) 141–146
Elsevier

BIOCHE 01472

A possible new mechanism of oxygen affinity modulation in mammalian hemoglobins

Clara Fronticelli

University of Maryland School of Medicine, Department of Biological Chemistry, 660 West Redwood Street, Baltimore, MD 21201, U.S.A.

Received 28 December 1989
Accepted 13 February 1990

Oxygen affinity; Hemoglobin; Chloride; Diphosphoglycerate; Binding mechanism

Bovine red cells do not contain appreciable amounts of 2,3-diphosphoglycerate (2,3-DPG). Bovine hemoglobin, however, has a particular sensitivity to chloride ions and as a result it can attain oxygen affinity values lower than those measured for human hemoglobin in the presence of 2,3-DPG. The interaction of bovine hemoglobin with anions is modulated by the hydrophobic characteristics of the protein. Comparison of the hydropathy plots of primate and ruminant hemoglobins indicates constant regions of opposite hydrophobicity, which have fixed amino acid differences. A model is proposed for explaining the regulation of oxygen affinity by chlorides, as an alternative to the classic modulation by 2,3-DPG.

1. Introduction

In human red cells, the interaction of 2,3-DPG with hemoglobin (Hb) regulates the efficient release of oxygen to the tissues [1]. Bovine red cells do not contain appreciable amounts of 2,3-DPG [2], however, human and bovine erythrocytes have the same oxygen affinity, indicating that either bovine Hb has an intrinsically low oxygen affinity, or that the oxygen affinity of this Hb becomes physiologically acceptable through a different mechanism of regulation.

In this laboratory, we have observed that, in the absence of organic and inorganic anions, the oxygen affinities of human and bovine Hbs were similar [3], indicating that the low oxygen affinity of bovine Hb could not be an intrinsic property of the molecule. Furthermore, we have shown that bovine Hb had a particular sensitivity to chlorides and, as a result, at physiological chloride concentration (0.1 M) it could attain oxygen affinity values lower than those measured for human Hb in the presence of 2,3-DPG [4]. The data indicated that the enhanced sensitivity of bovine Hb to the solvent components was due to the presence of extra chloride-binding sites, absent in human Hb. We have also seen that bovine Hb could discriminate between the halides on the basis of the charge density of the molecules [4], as if the interaction of the protein with the anions was modulated by the hydrophobic characteristics of the protein. In contrast, human Hb cannot discriminate between the halide atoms [4].

The different functional characteristics of human and bovine Hbs must originate from the amino acid compositions of the two proteins. According to Perutz and Imai [5], the presence in bovine Hb of a hydrophobic methionine at position NA1, replacing valine NA1 and histidine NA2 of human β-chains, produces a distortion of the A-helix which stabilizes the deoxy form of Hb.

Correspondence address: C. Fronticelli, University of Maryland School of Medicine, Department of Biological Chemistry, 660 West Redwood Street, Baltimore, MD 21201, U.S.A.

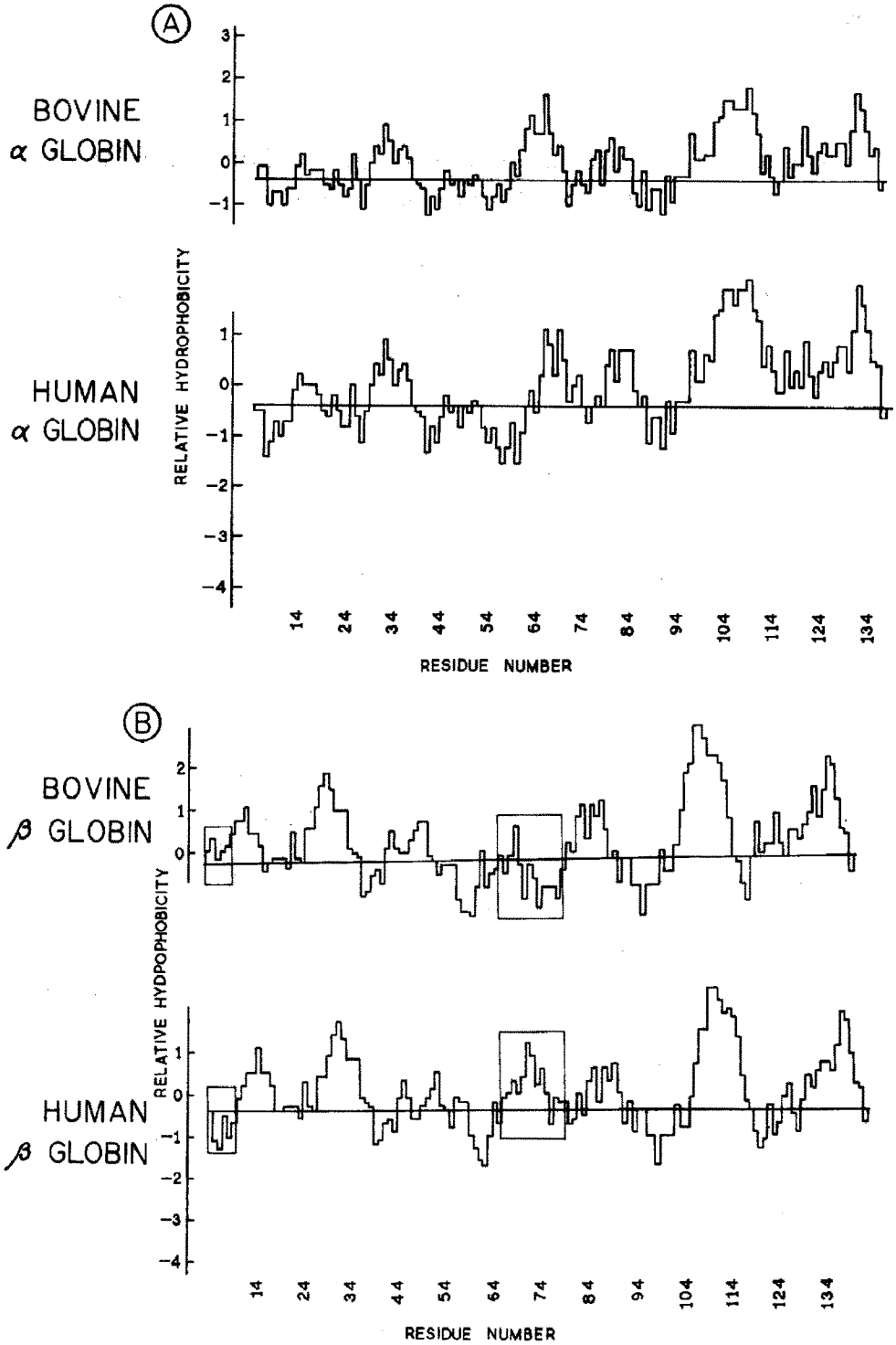

Fig. 1. (A) Hydropathy plots of bovine and human α-chains. (B) Hydropathy plots of bovine and human β-chains; the squares indicate the two regions of opposite hydrophobicity. These plots were obtained using the hydrophobicity values derived by Kite and Doolittle [6].

It can be proposed that the conformational change described by Perutz is correlated with the high sensitivity of bovine Hb to chlorides and with the hydrophobic regulation of the anion's interaction.

In order to acquire a better understanding of the relationship between the anion binding and hydrophobic characteristics of the Hb molecule, we have compared the hydropathy plots of primate and ruminant Hbs of which human and bovine Hbs are prototypes.

The result of this analysis indicates that in the β-chains of primate and ruminant Hbs, there are two constant regions of opposite hydrophobicity which have fixed amino acid differences. On the basis of these observations, a model has been formulated for explaining the regulation of oxygen affinity by chlorides, as an alternative to the classic modulation by 2,3-DPG.

2. Hydropathic characteristics of the hemoglobin molecule

The hydropathic characteristics of the α- and β-chains of human and bovine Hbs obtained using the hydrophobicity values derived by Kite and Doolittle [6] are shown in fig. 1. In the α-chains the hydropathy profile is very similar for the two Hbs, however, in the β-chains two regions of opposite hydrophobicity are evident, one comprising residues at the amino-terminal end, the other residues at the E helix and EF corner. The hydropathy plots of the α- and β-chains of the primate and ruminant Hbs listed in table 1 are very similar to the profiles obtained for human and bovine Hbs, respectively (fig. 1). The histogram of the values obtained from the two regions of opposite hydrophobicity in primate and ruminant β-chains is shown in fig. 2. The bars correspond to the average of the hydrophobicity values of each region, respectively, for the segment βNA1–A7 and βE8–EF5 in primate and ruminant β-chains.

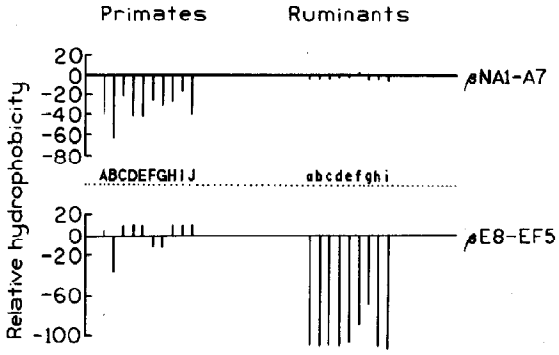

Fig. 2. Histogram of the hydrophobicity values calculated for the regions βNA1–A7 (top) and βE8–EF5 (bottom) of the β-chains of the primate and ruminant hemoglobins listed in table 1.

Table 1

List of primate and ruminant hemoglobins analyzed for their hydropathic characteristics

β-Globins analyzed	
Primates	Ruminants
(A) Human, chimpanzee, and gorilla	(a) Bovine
(B) Rhesus macaque, Japanese	(b) Gayal
(C) Spider monkey	(c) Yak
(D) Black and red tamarin	(d) Greater kudu
(E) Brown headed tamarin	(e) European moose
(F) White fronted capuchin	(f) Virginia white-tailed deer
(G) Brown capped capuchin	(g) Sheep
(H) Common gibbon	(h) Goat β-A chain
(I) Night monkey	(i) Goat, sheep β-C chain
(J) Common squirrel monkey	

Table 2

Hydrophobicity values of the regions NA1–A7 and E8–EF5 of primate and ruminant β-chains

The values are the average of 10 sequences for primates and 9 sequences for ruminants.

Hydrophobicity scale	Primates (Sequence)	Ruminants (Sequence)
	(βNA1–A7 [1–10])	(βNA1–A7 [1–9])
Kite and Doolittle	−3.34	−0.08
Composite	−3.93	3.18
OMH	−20.0	−13.0
	(βE8–F5 [64–81])	(βE8–EF5 [63–80])
Kite and Doolittle	0.150	−92.8
Composite	13.6	−25.0
OMH	−13.34	−47.0

Table 3

Constant amino acid substitutions in the regions βNA1–A7 and βE8–EF5 in primate and ruminant β-chains

β-Globin	
Primates (10 sequences)	Ruminants (9 sequences)
NA1 Valine	Methionine
NA2 Histidine	deleted
A2 Proline	Alanine
E13 Glycine	Aspartic acid
E14 Alanine	Serine
E19 Leucine	Methionine
E20 Alanine	Lysine

In order to determine whether these differences were consistent with the use of other hydrophobicity scales, a similar analysis was performed using either the composite scale of Eisenberg [7], which represents the mean of four different hydrophobicity scales, or the OMH (optimal matching hydrophobicity [8] scale, which was derived directly from the hydrophobicity of amino acids in the globin family. Table 2 shows the average hydrophobicity values of the various sets. Although, as expected, the absolute values differ, in all cases the analyses confirm the presence of different regions of hydrophobicity in primate and ruminant β-chains.

In order to correlate the different hydrophobicity of residues βNA1–A7 and βE8–EF5 with differences in amino acid composition, we compared the sequences of these residues in primate and ruminant β-chains. The results in table 3 show the presence of constant substitutions between primate and ruminant Hbs.

Another constant difference is the presence in ruminant β-chains of an arginine at position βHC1 replacing a lysine at that position in human Hb. Arginine is expected to be a better ligand for Cl^- than lysine because a guanidinium ion can form two hydrogen bonds to Cl^-, whereas an alkylammonium ion can form only one.

It is hypothesized that during the evolutionary process, these substitutions have been selected for introducing into ruminant Hbs an increased sensitivity to Cl^-, responsible for the lowered oxygen affinity of these Hbs.

3. Hydrophobic regulation of chloride binding

As originally proposed by Perutz and Imai [5], in bovine Hb a distortion of the A-helix produced by the change of hydrophobicity in the side chains of the amino acid residues in the segment βNA1–A7 forces these residues toward the interior of the molecule. This displacement is favored in the deoxy conformation of bovine Hb where the opening of the β-cleft exposes the N-terminal residues to water, while in the oxy conformation, where the space between the β-subunits narrows and water is expelled, the segment βNA1–A7 moves toward the central cavity. The presence of an alanine at position βA2 would increase the conformational mobility of the N-terminal residues. As indicated by Perutz, this phenomenon would be the principal factor in the modulation of oxygen affinity in ruminant Hbs.

The following two mechanisms are proposed, which associate the movement of the amino-terminal residues of the β-chains to the formation of the extra oxygen-linked chloride-binding sites pre-

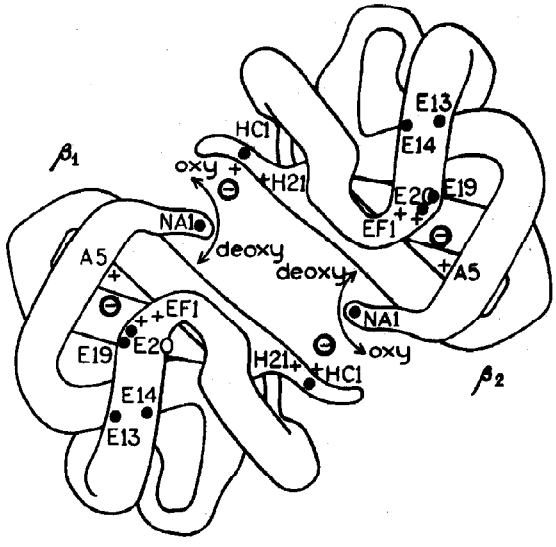

Fig. 3. The β-chains of human hemoglobin viewed down the two-fold symmetry axis. The full circles represent the positions of the constant amino acid substitutions between the β-chains of the primate and ruminant hemoglobins listed in table 1. The positive sign identifies the residues involved in the formation of the Cl^--binding sites. The chloride ions are represented by the open circles containing a negative sign.

sent in ruminant Hbs. They do not exclude each other. The rationale of this hypothesis is illustrated in fig. 3 which shows the two β-chains of human Hb viewed along the two-fold symmetry axis. The full circles represent the position of the consistent amino acid substitutions mentioned in table 3, and the positive signs identify the residues involved in the formation of the Cl^--binding sites. The chloride ions are themselves represented by the open circles containing a negative sign. The arrows at the N-terminal end suggest the movement of this portion of the A-helix in the transition between the oxy and deoxy conformation.

3.1. The anion-binding site is formed by βA5 Lys, βE20 Lys and βE21 His

The rationale for this binding site is as follows: in the transition to the deoxy form, the N-terminal residues move toward the interior of the protein, producing a distortion of the A-helix which, in turn, results in the displacement of βA5 Lys toward the E-helix. In ruminant Hbs, a positively charged lysine replaces an alanine at position E20 of human Hb. This substitution, together with the distortion of the A-helix, results in the formation of a cluster of three positively charged amino acid side chains, βA5 Lys, βE20 Lys, and βE21 His, which could comprise a Cl^--binding site. Binding of the Cl^- would be modulated by movement of the βA5 lysine brought about by the different positioning of the N-terminal end of the A-helix in the oxy and deoxy forms. In bovine hemoglobin the presence of hydrophilic residues at position βE13, βE14 and βE19 may help the formation of the cluster by contributing to the distortion of the E-helix in order to juxtapose correctly the three residues of the cluster.

3.2. The anion-binding site is at βHCl arginine

The rationale for this binding site is as follows: as discussed above, arginine is a better ligand for Cl^- than lysine. Arginine βHCl is positioned in the β-cleft, the same region where 2,3-DPG binds to human Hb. In the deoxy conformation the β-cleft is open, accessible to solvent components and arginine βHCl along with histidine βH21 form a Cl^--binding site. In the oxy form, as described by Perutz [9], the β-pocket closes and the solvent accessibility is reduced. As a result the hydrophobic segment βNA1–A7 moves away from the interior of the protein toward the center of the cavity, and further inhibits the access of Cl^- and water to the positively charged arginine and histidine.

4. Concluding remarks

This model proposes the presence in some mammalian hemoglobins of a molecular mechanism of regulation of oxygen affinity alternative to the classic regulation by 2,3-DPG. This new mechanism would be present in ruminant Hbs, however, a similar control could exist in other groups of mammalians, such as the feloidea, whose oxygen affinity is not modulated by 2,3-DPG [2]. In fact, unpublished data from this laboratory show that the oxygen affinity of cat Hb has a sensitivity to Cl^- similar to that of bovine Hb. There are two types of cat Hb, A and B; in both cases the hydrophobicity of the N-terminal residues of the β-chains is increased and a lysine is present at position βE20.

Studies are in progress to determine the validity of this model by introducing site-directed mutations into the β-chains of human Hb.

Acknowledgements

I am very grateful to Drs William Brinigar, Enrico Bucci and Anna Razynska for valuable discussions. Dr. William Brinigar brought to my attention the consistent presence in ruminant β-chains of an arginine at position βCH1. This research was supported by NIH Grants HL13164 and HL33629.

References

1 R. Benesch and R.E. Benesch, Nature 221 (1969) 618.
2 H.F. Bunn, Science 172 (1971) 1049.
3 C. Fronticelli, E. Bucci and C. Orth, J. Biol. Chem. 259 (1984) 10841.

4 C. Fronticelli, E. Bucci and A. Razynska, J. Mol. Biol. 202 (1988) 343.
5 M.F. Perutz and K. Imai, J. Mol. Biol. 136 (1980) 183.
6 J. Kite and R.F. Doolittle, J. Mol. Biol. 157 (1982) 105.
7 D. Eisenberg, R.M. Weiss, T.C. Terwillinger and W. Wileox, Faraday Symp. Chem. Soc. 17 (1982) 109.
8 R.M. Sweet and D. Eisenberg, J. Mol. Biol. 171 (1983) 479.
9 M.F. Perutz, Nature 228 (1970) 726.