KAWASAKI MED. J. VOL. 12 : NO. 2, 67-76 (1986)

Hemoglobinopathies Due to Abnormal Functional Properties of Hemoglobin Molecule

Part II. Stable Abnormal Hemoglobins with Increased Oxygen Affinity, Frequently Causing Polycythemia

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Accepted for Publication on June 5, 1986

ABSTRACT. In the first part of this review article stable abnormal hemoglobins of high oxygen affinity causing erythrocytosis were dealt with. About a half of them are due to mutation in the $\alpha_1\beta_2$ contact region of hemoglobin molecule, and the remaining half are mutational products originating in $\alpha_1\beta_1$ contact, heme contact, 2,3 DPG binding site, etc.

In the present paper, which forms the second part, stable variants of high oxygen affinity with mutations in the carboxy terminal segment, which cause either erythrocytosis or not, and the stable high oxygen affinity variants, the mutation of which involve $\alpha_1\beta_2$ contact without association of erythrocytosis are described.

In addition, instances of stable hemoglobin variants of increased oxygen affinity which do not lead to overt clinical symptoms are also discussed.

Key words : Hemoglobinopathies — Hemoglobins with altered oxygen affinity — Erythrocytosis — Cyanosis

3. *HEMOGLOBIN VARIANTS WITH MUTATIONS IN THE CARBOXY TERMINAL SEGMENT*

The carboxy terminal segments of both α and β chains contribute greatly to the overall stability of deoxy hemoglobin.[3] In the deoxy configuration, the tyrosine residue HC2 of both the α and β chains are wedged firmly between their F and H helices. Both the C terminal residue of α chain Arg 141 α_1 (HC3) and β chain His 146 β_1 (HC3) are involved in the salt bridges and all the interactions are oxygen linked. During oxygenation when the side chain of tyrosine is expelled from its crevice it pulls the carboxy terminal with it which causes a gradual breaking of the salt bridges between the two α chains [between the carboxy group of Arg 141 α_1 (HC3) and the α amino acid group of Val 1 α_2 (NA1) and between the guanidinium group of Arg 141 α_1 (HC3) and the carboxyl group of Asp 126 α_2 (H19)] and between the α and β chains [between the carboxyl group of His 146 β_1 (HC3) and the ε-amino group of

Lys 40 α_2 (C5) and between the imidazole group of His 146 β_1 (HC3) and the γ carboxyl group of Asp 94 β_1 (FG1)]. There is a total of eight of these bonds which are responsible for the unique properties of deoxyhemoglobin. Rupture of these salt bridges converts the spring loaded deoxy Hb into the relaxed (R) oxyhemoglobin. In addition, His β143 (H21) is also a binding site for 2,3 DPG, thus it will not be surprising that any substitution involving the C terminal residues will produce a hemoglobin variant with abnormal functional properties, decreased heme-heme interaction and Bohr effect and impaired interaction with 2,3 DPG.

Presently there are 18 variants with mutations in the carboxy terminal segment of both α and β chains (Table 4). Of these 18 variants only 15 mutants reported to have an increased oxygen affinity and 12 have a clear evidence of producing erythrocytosis (Table 5). Five replacements have been reported for the residue α141 (HC3) ; these include Hb Singapore α141 (HC3) Arg→Pro[36] ; Hb Suresnes α141 (HC3) Arg→His[48] ; Hb J Cubujuqui α141 (HC3) Arg→Ser[49] ; Hb Legnano α 141 (HC3) Arg→Leu[50] and Hb J-Camaguey α141 (HC3) Arg→Gly.[51] In the heterozygous state Hb Singapore does not manifest any clinical abnormality, while Hb Suresnes, Hb J Cubujuqui and Hb J-Camaguey are reported to produce a mild erythrocytosis. These substitutions

TABLE 4. Hemoglobin variants with mutations in the carboxy terminal

α Chain Variants

H Helix ——→← HC - Terminus		
136 - 137 - 138 - 139 - 140 - 141		
Ser - Lys - Tyr - Arg		
Thr		Tokoname
	Pro	Singapore (U)
	His	Suresnes
	Ser	J Cubujuqui
	Leu	Legnano*
	Gly	J-Camaguey (U)

β Chain Variants

H Helix ——→← HC-Terminus						
141 - 142 - 143 - 144 - 145 - 146						
His - Lys - Tyr - His						
Arg						Abruzzo*
Gln						Little Rock*
Pro						Syracuse*
	Asn					Andrew Minneapolis*
		His				Bethesda*
		Cys				Rainier*
		Asp				Fort Gordon*
		Term				McKees Rock
					Asp	Hiroshima*
					Pro	York*
					Arg	Cochin-Port Royal (N)
					Leu	Cowtown*

N = Normal Oxygen Affinity
U = Unknown Oxygen Affinity
* = Erythrocytosis

TABLE 5. Variants involving carboxy terminal residues producing erythrocytosis

No.	S. Variant Substitution	Position in Molecule	RBC (10^{12}/L)	Hb (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Retics (%)	Abn. Hb(%)	Race	Elect. Mobility	O_2 Affinity	n	Bohr Effect	Ref.
1.	Legnano α141(HC3)Arg→Leu	E,deoxy	6.2	20	58	92	32	34		34.4	Italian	Like J	Increased	↓		50
2.	Abruzzo β143(H21)His→Arg		7.5	14.0						94.0?	Italian	Between F & Lepore	Increased	Normal	Normal	52
3.	Little Rock β143(H21)His→Gln	Same			70					50-55	American	Between A and F	Increased	Normal	Normal	53
4.	Syracuse β143(H21)His→Pro	Same		19.2-23.8	60					50.0	American	Slower than A	Increased	↓	Reduced	54
5.	Andrew-Minneapolis β144(HC1)Lys→Asn	E	5.9	19.8	57				1.5	>50.0?	American	Faster than A Like A_3	Increased	↓	Normal	55
6.	Bethesda β145(HC2)Tyr→His			20.5	55					45.0	Chinese	Like A	Increased	↓	Reduced	57
7.	Rainier β145(HC2)Tyr→Cys			21.0	59					30.0	American	Like A	Increased	↓	Reduced	57
8.	Fort Gordon β145(HC2)Tyr→Asp		7.0	21.2	69				1.8	30.0	Black American	Faster than A	Increased	↓	Normal	58
9.	McKees Rocks β145(HC2)Tyr→Term			17.8	55					46.0	American	Like A	Increased	Absent	Reduced	59
10.	Hiroshima β146(HC3)His→Asp			17.4	55					50.0	Japanese	Faster than A	Increased	Normal	Reduced	60, 61
11.	York β146(HC3)His→Pro		mild							50.0	Caucasian	Slower than A	Increaeed	↓	Reduced	62
12.	Cowtown β146(HC3)His→Leu		5.8	18.9	51	88	33	37		45.0	American	Like A	Increased		Reduced	64

of Arg α141 (HC3) either by Prolyl residue (Hb Singapore) or by Histidyl residue (Hb Suresnes) or by Seryl residue (Hb J Cubujuqui) or by Leucyl residue (Hb Legnano) or by Glycyl residue (Hb J-Camaguey) can have profound effect on the functional properties of the Hb molecule as Arg α 141 is one of the residues involved in the stabilization of the deoxy form of hemoglobin molecule ; However, no functional data is available for both Hb Singapore and J-Camaguey, but remaining three have an increased oxygen affinity and normal Bohr effect.

Three variants namely Hb Abruzzo β143 (H21) His→Arg,[52] Hb Little Rock β143 (H21) His→Gln[53] and Hb Syracuse β143 (H21) His→Pro[54] involve the replacement of His β 143 which is one of the binding sites for 2,3 DPG. Hb Abruzzo β143 (H21) His→Arg has a high oxygen affinity but the cooperativity between the subunits and Bohr effect is normal. It will be interesting to note that Hb Abruzzo accounts for 92-94% of the total hemoglobin. A high level of 2,3 DPG was found in the red cells of the patients and the tissue hypoxia was responsible for the erythrocytosis. In Hb Little Rock β143 (H21) His→Gln, it has been suggested that the glutaminyl residue forms a new hydrogen bond with Asn β139 (H17) of the other β chain, thus contributing to the stability of molecule in the oxy conformation. The same effect, i.e. loss of 2,3 DPG binding site, has been observed for Hb Syracuse.

In Hb Andrew-Minneapolis β144 (HCl) Lys→Asn[55] a new hydrogen bond is formed between the carbonyl oxygen of the asparaginyl residue β144 (HC1) and imidazole portion of the carboxy terminal histidyl residue β146 (HC3) which restrict the movement of the phenolic side chain of the penultimate tyrosyl residue β145 (HC2). The purified mutant exhibits nearly normal heme-heme interaction and Bohr effect is decreased by 50%. Another abnormal hemoglobin with an increased oxygen affinity known as Hb Tokoname α141 (HCl) Lys→Thr,[56] with a substitution at position 141 of the α chain which corresponds with position 145 of the β chain has been reported.

Four other mutants, Hb Bethesda β145 (HC2) Tyr→His[57]; Hb Rainier β145 (HC2) Tyr→Cys[57]; Hb Fort Gordon β145 (HC2) His→Asp[58] and Hb McKees Rock β145 (HC2) His→Term[59] have a substitution of tyrosyl residue β145 (HC2) which is an important determinant in the transition of the oxy state to the deoxy state of hemoglobin molecule and vice versa.[3] Any charged residue, such as the histidyl residue in Hb Bethesda or aspartic acid in Hb Fort Gordon can not occupy the pocket and would interfere with the formation of salt bridges by the adjacent residue in the terminal position which normally links the subunit in the deoxy conformation. As a result the ability of the variant to form a normal deoxy structure is drastically reduced and its oxygen affinity greatly increased. Hb Rainier also involves the replacement of tyrosyl β145 (HC2) residue by a cysteinyl. This Hb has an increased alkali resistance like Hb F, resulting from the formation of bonds between the hemoglobin tetramer. Hb McKees Rock, which is the result of a "non-sense" point mutation at the codon for Tyr β145 is the first example in which the last two residues (β145, 146) of the carboxy terminal have been deleted thus shortening the β globin chain. This hemoglobin has the highest oxygen affinity of any variant that has been reported. Its Bohr effect is reduced and is devoid of subunit cooperativity.

There are four examples in which the β terminal residue His β 146 (HC3)

is replaced. These include Hb Hiroshima β 146 (HC3) His→Asp[60,61]; Hb York β146 (HC3) His→Pro[62]; Hb Cochin-Port Royal β146 (HC3) His→Arg[63] and Hb Cowtown β146 (HC3) His→Leu.[64]

Hb Hiroshima has a decreased Bohr effect which is attributed to the substitution of aspartate for histidine at position β146 (HC3). This replacement results in destabilization of the deoxy configuration due to loss of the intra chain salt bridge between the histidyl residue at position β146 and the aspartyl residue at position β94. An altered C-terminal salt bridges are responsible for significantly increased oxygen affinity, reduced cooperativity between the subunits and reduced Bohr effect of Hb York. Hemoglobin Cochin Port-Royal has a normal p_{50} and n values suggesting that most of its salt bridges are still intact. As stated earlier, β146 (HC3) involved in the formation of salt bridges that accomplish stabilization of the deoxy conformation and contribute to the Bohr effect. The replacement of His by Leu as in Hb Cowtown interferes with the formation of these salt bridges.

4. *STABLE HEMOGLOBIN VARIANT INVOLVING $\alpha_1\beta_2$ CONTACT BUT WITHOUT ERYTHROCYTOSIS*

The category includes only 6 abnormal hemoglobins all of which involve $\alpha_1\beta_2$ contact, have an increased oxygen affinity but without any clinical manifestations (Table 6). These variants are Hb G-Georgia α95 (G2) Pro→Leu[28]; Hb Rampa α95 (G2) Pro→Ser[29]; Hb Denmark Hill α95 (G2) Pro→Ala[30]; Hb Hirose β37 (C3) Trp→Ser[65]; Hb Athens-Ga β40 (C6) Arg→Lys[66] and Hb Austin β40 (C6) Arg→Ser.[67]

Hemoglobin St. Lukes α95 (G2) Pro→Arg[31] also involves the substitution of Pro α95, but this variant has decreased oxygen affinity. Proline α95 (G2) forms a non polar bond with β37 Trp (C3) in both the deoxy and oxy conformation. When this residue is replaced by Leucyl (Hb Georgia) or by Seryl (Hb Rampa) or by Alanyl (Hb Denmark Hill) or by Arginyl (Hb St. Lukes), the oxy derivatives dissociate into dimers and reassociate into tetramer when the oxygen is removed. Apparently these hydrophobic contacts are of great importance for the stability of the $\alpha_1\beta_2$ interface in the oxy state. The decreased heme-heme interaction for these variants can be readily explained by the decreased dissociation of the oxy variants.

Trp β37 (C3) is in contact with arginyl residue α92 (FG4) which effects heme-heme interaction via the $\alpha_1\beta_2$ contact. This Trp→Ser substitution in Hb Hirose, destabilizes the deoxy conformation. Hb Hirose like other variants at $\alpha_1\beta_2$ contact does not produce erythrocytosis but individuals with this variant have a high oxygen affinity.

Hemoglobin Athens-Ga (β40 Arg→Lys) was discovered in a Caucasian student and his three family members, whereas Hb Austin (β40 Arg→Ser) was found in three other unrelated heterozygotes of Mexican origin. Both these variants involve $\alpha_1\beta_2$ contact and have an increased oxygen affinity. β40 (C6) Arg makes contact with α91 (FG3) Arg, α92 (FG4) Leu, α41 (C6) Thr and α42 (C7) Tyr in the oxy conformation. But in the deoxy conformation, it makes only contact with α92 (FG4) Arg and α42 (C7) Tyr. Through these contacts, the β40 (C6) Arg participates in the shortest bridge between any pair of heme groups in the hemoglobin molecule. Therefore,

TABLE 6. Stable human hemoglobin variants with increased oxygen affinity involving $\alpha_1\beta_2$ contact but without erythrocytosis

No.	S. Variant Substitution	Position in Molecule	RBC (10^{12}/L)	Hb (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Retics (%)	Abn. Hb(%)	Race	Elect. Mobility	O_2 Affinity	n	Bohr Effect	Ref.
1.	G–Georgia α95(G2) Pro→Leu	CC	4.5	10.8	35				0.6	23.0	Black American	Slower than A	Increased	↓		28
2.	Rampa α95(G2) Pro→Ser	Same								23–48	Indian	Slower than A	Increased	↓		29
3.	Denmark Hill α95(G2) Pro→Ala	Same	3.9	12.0		87	30			20–30?	West Indian	Slower than A	Increased	↓	Reduced	30
4.	Hirose β37(C3) Trp→Ser	I	4.8	14.4	43				0.9	41.0	Japanese	Between S & F	Increased	↓	Reduced	65
5.	Athens–Ga β40(C6) Arg→Lys	E	4.8	14.0	39	82				49.6	American	Slower than A	Increased	Normal	Normal	66
6.	Austin β40(C6) Arg→Ser	Same	———			Normal		———		45.0	Mexican	Between A & F	Increased	↓	Normal	67

TABLE 7. Others—without any clinical manifestations

No.	S. Variant Substitution	Contact	Position in Molecule	RBC (10^{12}/L)	Hb (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Retics (%)	Abn. Hb(%)	Race	Elect. Mobility	O_2 Affinity	n	Bohr Effect	Ref.
1.	Sawara α6(A4) Asp→Ala	SB to Lys(H10)	E	Normal							17.0	Japanese	Slower than F	Increased	Normal	Normal	68,69
2.	Dunn α6(A4) Asp→Asn	Same	Same	Normal							11.6	Black American	Like F	Increased			70
3.	Ferndown α6(A4) Asp→Val	Same	Same		7.7						8.0	British	Behind A	Increased			71
4.	Woodville α6(A4) Asp→Tyr	Same	Same		13.3		84.9	29.7			9.0	Vietnamese	Like F	Increased			72
5.	Evanston α14(A12) Trp→Arg		I	6.3	10.7	38	60	17.3	29		7.1	Black American	Like S	Increased	Normal	Normal	73,74
6.	G-Norfolk α85(F6) Asp→Asn		E	4.7	14.9		89	32		2.7	15.0	English French	Slower than A	Increased	Normal	Normal	75,76
7.	Atago α85(F6) Asp→Tyr		Same								22.0	Japanese	Slower than F	Increased	↓		77
8.	Inkster α85(F6) Asp→Val		Same			45		slt. polychrom.			22.0	English German		Increased			78
9.	Dallas α97(G4) Asn→Lys	Heme		5.3	14.5	44					23.0	American	Between A and F	Increased		Normal	79
10.	Tarant α126(H9) Asp→Asn	$\alpha_1\beta_1$	CC, SB to Arg(HC3)	Normal							19.5	Mexican American	Between F and S	Increased	↓	Normal	80
11.	Tokoname α139(HC1)Lys→Thr		E	4.9	15.6	46	93	31	34	0.7	24.8	Japanese	Like J	Increased	↓	Normal	56
12.	Suresnes α141(HC3)Arg→His	SB	E, deoxy	5.5	15.9	43.9	81	28.5	36		39.0	French	Like J	Increased	↓	Decreased	48
13.	J Cubujuqui α141(HC3) Arg→Ser	Same	Same		15.1	46					36.0	Costarican	Like J	Increased	↓	Decreased	49
14.	Deer Lodge β2(NA2) His→Arg	2,3 DPG Binding	IC	3.9	12.6	36				1.2	40.0	Welsh-Dutch	Slower than A	Increased			81
15.	Okayama β2(NA2) His→Gln	Same	Same	3.97	13.9	38.5	97	35.0		0.9	47.4	Japanese	Like A_{1C}	Increased			82
16.	Porto Alegre β9(A6) Ser→Cys		E									Brazilian		Increased	↓	Normal	83,84
17.	G-His-Tsou β79(EF3) Asp→Gly	SB to Lys β8(A5)	E, deoxy	No anemia							46.0	Chinese	Like G	Increased	Normal	Increased	85,86

the replacement of arginyl residue either by a lysyl residue as in Hb Athens-Ga or by Seryl in Hb Austin can alter the oxygen binding properties of the hemoglobin molecule.

5. *OTHER HEMOGLOBIN MUTANTS INVOLVING DIFFERENT CONTACTS WITHOUT ANY CLINICAL MANIFESTATIONS*

Several hemoglobins have been listed in this group (Table 7). Although all of them have a raised oxygen affinity but neither of them is associated with anemia nor hematological abnormalities. Four hemoglobin variants involving residue α6 Asp have been described : Hb Sawara α6 (A4) Asp→Ala[68,69]; Hb Dunn α6 (A4) Asp→Asn[70]; Hb Ferndown α6 (A4) Asp→Val[71] and Hb Woodville α6 (A4) Asp→Tyr.[72] The α6 (A4) Asp forms a salt bridge with α127 (H10) Lys of the same α chain and when this residue is replaced by one of the above listed amino acids, the $\alpha_1$6 - $\alpha_1$127 salt bridge does not form thus favoring the R structure of the tetramer and increased oxygen affinity.

Hb Evanston α14 (A12) Trp→Arg[73,74] with thalassemia like expression was found in two unrelated blacks. This variant involves the substitution of α14 (A12) Trp, a hydrophobic residue which is located internally. Replacement of this large aromatic tryptophan by a charged arginine residue will disrupt the structural relationship in this area since arginyl residue will tend to be located on the external surface of molecule. This substitution of Trp→Arg is more favorable to the formation and stability of the R state, giving rise to an increased oxygen affinity.

The high oxygen affinity of Hb G-Norfolk α85 (F6) Asp→Asn[75,76] ; Hb Atago α85 (F6) Asp→Tyr[77] and Hb Inkster α85 (F6) Asp→Val[78] is difficult to explain. The residue α85 (F6) Asp occupies an external position which is neither involved in a subunit contact nor in a heme contact. It has been suggested that Asp α85 might be involved in an interaction with His α89 which lies just below it in the next turn of helix. Hb Dallas α97 (G4) Asn→Lys[79] is another stable variant involving heme contact with an increased oxygen affinity, but without producing erythrocytosis. The residue α97 (G4) Asn normally interact with α99 (G1) Asp to stabilize the deoxy or T conformation. An alteration of this stabilizing affect probably explains the high oxygen affinity of Hb Dallas. Hb Tarant α126 (H9) Asp→Asn[80] has substitution of asparagine for anaspartyl residue at position 126, one of the site involved in the $\alpha_1\beta_1$ contact. In Hb A Asp α126 forms a hydrogen bond with β35 (C1) tyrosine. It is also linked to β34 valine by a non polar bond. In addition to that in the deoxy conformation Asp α126 forms a noncovalent electrostatic salt bridge with Arg α141 (HC3). This substitution of Asp→Asn in Hb Tarant affects the deoxy or T state of the molecule as the above contacts cannot be formed, accounting for the high oxygen affinity and low heme-heme interaction between the subunits.

As stated repeatedly, an amino acid substitution affecting the residues involved in 2, 3 DPG binding would indirectly can cause a raised oxygen affinity. The substitution of an arginyl residue for histidine at β2 as in Hb Deer Lodge β2 (NA2) His→Arg[81] and or by glutamine in Hb Okayama β2 (NA2) His→Gln[82] affects both the Kinetics and equilibria of ligand binding. As a result of these amino acid substitution, the grip of the two β chains

on the metabolite 2, 3 DPG is weakened, encouraging the oxy structure.

Hb Porto Alegre β9 (A6) Ser→Cys[83,84] is a variant that polymerizes by the formation of intermolecular disulfide bonds between the extra cysteinyl residue at the β9 position, however, its oxygen binding properties are unaffected by the polymerization. Both the tetramer and disulfide polymers have oxygen affinities somewhat higher than normal hemoglobin, reduced cooperativity between the subunits and normal Bohr effect. Hb G-His-Tsou β79 (EF3) Asp→Gly[85,86] is a mutant with substitution in the EF bend of β chain. In Hb G-His-Tsou a deoxy salt bridge is missing between β79 Asp (EF3) and β8 (A5) on the same chain, thus accounting for the increased oxygen affinity of the variant. (To be continued to the third part)

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