Hemoglobinopathies Due to Abnormal Functional Properties of Hemoglobin Molecule
Part III. Stable and Unstable Abnormal Hemoglobins with Altered Oxygen Affinity

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ABSTRACT. Stable abnormal hemoglobins of high oxygen affinity causing erythrocytosis and those unassociated with erythrocytosis were dealt with in the first and the second parts of this review article.
    In the present paper, which forms the third and the last part, hemoglobin variants with altered oxygen affinity which cause either cyanosis or not, and unstable hemoglobins with altered oxygen affinity are described.

Key words : Hemoglobinopathies —
                    Hemoglobins with lowered oxygen affinity —
                    Unstable hemoglobins with altered oxygen affinity

HEMOGLOBIN VARIANTS WITH DECREASED OXYGEN AFFINITY

The observed abnormalities of hemoglobin function are related to the site of the amino acid substitution. Perutz and his co-workers[3,10] have demonstrated that oxygen affinity is altered when the amino acid substitution affects the oxygen binding by shifting the allosteric equilibrium between the oxy and deoxy configuration of the Hb molecule. As a rule, any amino acid substitution that tend to favor the deoxy conformation will result in a hemoglobin with decreased oxygen affinity. Only 13 stable hemoglobin variants (Table 8) have been reported in which a reduced oxygen affinity occurs as the sole abnormality. In addition to that a number of the unstable hemoglobins have also been found to have decreased oxygen affinity (Table 9). However, it might be expected that because of increased oxygen supply resulting from the low oxygen affinity, the erythropoietic response might be reduced and the variant would be associated with mild anemia. But this is not the case and the clinical association of anemia with low oxygen affinity is not clearly established as the association of erythrocytosis with increased oxygen affinity. Only few variants like Hb Agenogi, Hb St. Mande exhibit a mild anemia and all the rest have normal hemoglobin levels.

Hb Raleigh $\beta 1$ (NA1) Val$\rightarrow$Ac-Ala[87] is unique in which the N terminal valyl residue of β chain has been replaced by acetylated alanyl residue. The only other example of blocked β chain is glycosylated hemoglobin, Hb A_{1C}.

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TABLE 8. Hemoglobin with decreased oxygen affinity.

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	O₂ Affinity	n	Bohr Effect	Ref.
1.	St-Lukes α95(G2) Pro→Arg	α₁β₂	CC		14.5	46				1.0	10.1	Maltese	Like A	Decreased	↓		105
2.	Raleigh β1(NA1) Val→AcAla	DPG Binding oxy;SB to HC3 (146)	IC	⟵——— Normal ———⟶							45.0	American	Faster than A	Decreased	Normal	Normal	87
3.	Connecticut β21(B3) Asp→Gly		E		13.0	37	80				40.0	Polish American	Like S	Decreased	Normal	Normal	88
4.	Rothschild β37(C3) Trp→Arg	α₁β₂	I									French?	Between A and S	Decreased	↓		89
5.	Bologna β61(E5) Lys→Met		E, SB to Asp(B3)									Italian	Faster than A	Decreased	Normal	Normal	90
6.	Vancouver β73(E17) Asp→Tyr		E	6.4	12.2	39	61	19.2	31.5	2.8	89.5	Chinese	Like G	Decreased	Normal	Increased	92
7.	Korlebu β73(E17) Asp→Asn		Same								50.0	Ghanaian	Like D	Decreased			93
8.	Mobile β73(E17) Asp→Val		Same	5.3	14.0	43	81	26.0	32.0		32.5	Black American	Like S	Decreased	Normal	Normal	94
9.	Providence β82(EF6) Lys→Asn →Asp	2,3 DPG Binding Site	SCC								19.3 (Asn) 32.3 (Asp)	American	Faster than A	Decreased	Normal	Decreased	106
10.	Agenogi β90(F6) Glu→Lys		E								44.5	Japanese	Like E	Decreased	Normal	Decreased	95
11.	Kansas β102(G4) Asn→Thr	α₁β₂ heme, oxy			13.9						51.0	American	Slower than A	Decreased	↓	Normal	97
12.	St. Mande β102(G4) Asn→Tyr	Same		3.9	11.8	36	91	30.0	33.0	1.0	38.0	French	Like F	Decreased			98
13.	Yoshizuka β108(G10) Asn→Asp	α₁β₁	IC	4.1	14.7	37				0.7	50.0	Japanese	Slightly Faster than A	Decreased	Normal	Reduced	99

E=External   I=Internal   SB=Salt bridge   CC=Central cavity   IC=Internal cavity

SCC=Surface of central cavity   SP=Surface pocket

In Hb A, the α amino group of the two $\beta 1$ valines are among the eight positively charged residues involved in the binding of organic phosphates and small anions. Substitution of acetylalanine for valine reduces the number of cationic groups to six resulting in decreased oxygen affinity and decreased interaction with organic phosphates.

The residue $\beta 21$ (B3) Asp, is an external residue; it is not involved in any of the contact which are of critical importance to the structure or function of Hb molecule. The decreased oxygen affinity of Hb Connecticut $\beta 21$ (B3) Asp→Gly[88] may be due to loss of salt links between $\beta 21$ (B3) Asp and $\beta 65$ (E9) Lys and possibly $\beta 61$ (E5), which hold the E helix in its proper position. Trp $\beta 37$ (C3) is in contact with arginyl residue $\alpha 92$ (FG4); the substitution of Trp→Arg as in Hb Rothschild $\beta 37$ (C3) Trp→Arg[89] favors the deoxy T configuration, whereas, this Trp→Ser substitution in Hb Hirose stabilizes the tetramer in the oxy configuration producing a variant with an increased oxygen affinity.

Hb Bologna $\beta 61$ (E5) Lys→Met[90] was found in association with β thalassemia in a 11-year-old Italian child. The residue $\beta 61$ (EF5) Lys is an external one; it is close to the distal His E7, which forms a salt bridge with aspartyl residue $\beta 21$ (B3) of the same chain and is also in contact with the side chains of the residue $\beta 25$ (B7) Gly, $\beta 55$ (D6) Met and $\beta 56$ (D7) Gly. The replacement of the charged polar lysine by the non hydrophobic methionine can affect some of these contacts bringing some distortion in the conformation of molecule in the E-B contact area because of tendency of methionine to depart from the hydrophobic external environment.

Four different mutants have been reported with a substitution at $\beta 73$. These are Hb C-Harlem $\beta 6$ (A3) Glu→Val and $\beta 73$ (E17) Asp→Asn[91]; Hb Vancouver $\beta 73$ (E17) Asp→Tyr[92]; Hb Korle-Bu $\beta 73$ (E17) Asp→Asn[93] and Hb Mobile $\beta 73$ (E17) Asp→Val.[94] Except for Hb C-Harlem, all of the $\beta 73$ substituted hemoglobins thus reported so far exhibit a decreased oxygen affinity[95] and no clinical abnormalities have been ascribed to these $\beta 73$ substitutions. In each of above four mutants, a negatively charged aspartyl residue is replaced by a neutral or uncharged amino acid and decrease in oxygen affinity varies proportionately with the size of the substituted residue at $\beta 73$. This $\beta 73$ (E17) residue is not a heme contact site and Wajcman and Jones have suggested[95] that the changes in oxygen affinity result from the formation of abnormal van der Waals forces between the abnormal residues and the side chain of residue $\beta 84$ in the deoxy conformation.

In Hb Agenogi $\beta 90$ (F6) Glu→Lys,[96] tyrosyl residue is retained in its deoxy position in its pocket by a linkage formed between the lysine and C terminal carboxyl group of the same β chain accounting for the low oxygen affinity of this variant.

Asparaginyl residue $\beta 102$ (G4) is one of the invariant residue. In the oxy configuration Asn forms a hydrogen linkage with Asp $\alpha 94$ (G1) in the $\alpha_1 \beta_2$ contact. A substitution of this Asn by Thr is reported in Hb Kansas $\beta 102$ Asn→Thr[97] and the same Asn has been replaced by Tyrosyl in Hb St. Mande $\beta 102$ Asn→Tyr.[98] In the oxy conformation, the Asn $\beta 102$ (G4) is hydrogen bonded across $\alpha_1 \beta_2$ contact to Asp $\alpha 94$ (G1). A substitution of this $\beta 102$ asparagine by threonine in Hb Kansas breaks this oxy conformation bond but presumably forms another hydrogen bond with the carboxyl group of Asp $\beta 99$

(G1) of the same chain thus favoring the deoxy conformation. The same argument has been used for the low oxygen affinity of Hb St. Mande. Any interference with hydrogen bond formation between Asn $\beta102$ (G4) and Asp $\alpha94$ (G1) would shift the $R \rightleftharpoons T$ equilibrium towards the T state, resulting in a lower oxygen affinity. Both Hb Kansas and St. Mande are associated with marked decrease in oxygen affinity and cause cyanosis in the carrier. Hb Kansas is interesting as the oxygen affinity is so low that at normal arterial oxygen tensions, there is sufficient desaturation to give rise to clinical cyanosis. It seems likely that with such a low oxygen affinity, oxygen uptake in the lungs is so impaired that to maintain adequate tissue oxygenation. Even when Hb concentration is normal approximately 3/dl is non-functioning physiologically, as the arterial saturation is about 75%.

Hb Yoshizuka $\beta108$ (G10) Asn$\rightarrow$Asp[99] was found in a Japanese. This variant has also decreased oxygen affinity but less marked than Hb Kansas. The Asn $\beta108$ (G10) lies in the internal cavity and its main chain carboxyl forms hydrogen bond to His $\alpha103$ (G10). It seems likely that the introduction of a charged group into central cavity might result in interaction between the polar groups and the substituted side chains disrupting the hydrogen bonds which contribute to the stability of the contacts between $\alpha\beta$ subunits.

UNSTABLE HEMOGLOBINS WITH AN ALTERED OXYGEN AFFINITY

More than 40% of the total unstable hemoglobin variants, have either increased or decreased oxygen affinity (Table 9).[100~102] The Figures 3 and 4 denotes the position of both α and β chain variants in their respective helices: Most of these unstable variants either have substitution at $\alpha_1\beta_1$ and $\alpha_1\beta_2$ contacts; in central cavity; at heme contact; at external surface or with deletion of one or more amino acid residues. These amino acid substitutions in the area of heme pocket and along the contacts between the α and β subunits are liable to give rise to abnormal hemoglobins with altered oxygen affinity. It is therefore, follows that many hemoglobins that were classified primarily as

TABLE 9.   Unstable hemoglobin mutants with altered oxygen affinity.

α-Chain variants   (See Figure 3)
   Hb Kariya; Hb Torino($\downarrow$); Hb Kokura; Hb Etobicoke; Hb Moabit($\downarrow$);
   Hb Setif ($\downarrow$); Hb Hopkins-II.

β-Chain variants (See Figure 4)
   Hb Belfast; Hb Strasbourg; Hb Miyashiro; Hb |Palmerston North; Hb
   Riverdale-Bronx; Hb Moscva; Hb St. Louis; Hb Genova; Hb Philly;
   Hb Hazebrouck; Hb Hammersmith($\downarrow$); Hb Cheverly($\downarrow$); Hb Okaloosa
   ($\downarrow$); Hb Williamette; Hb Duarte; Hb Zürich; Hb J-Calabria; Hb Seattle($\downarrow$);
   Hb Shepherds Bush; Hb Pasadena; Hb Baylor; Hb Buenos Aires; Hb
   Caribbean($\downarrow$); Hb St. Etienne; Hb Mozhaisk; Hb Okazaki; Hb Nagoya;
   Hb Nottingham; Hb Beth Israel($\downarrow$); Hb Southampton, Hb Tübingen;
   Hb Burke($\downarrow$); Hb Peterborough($\downarrow$); Hb Crete; Hb Altdorf; Hb Hope;
   Hb Toyoake; Hb Köln.

Deleted residues
   Hb Leiden; Hb Lyon; Hb Niteroi($\downarrow$); Hb Tours; Hb Gunn Hill.

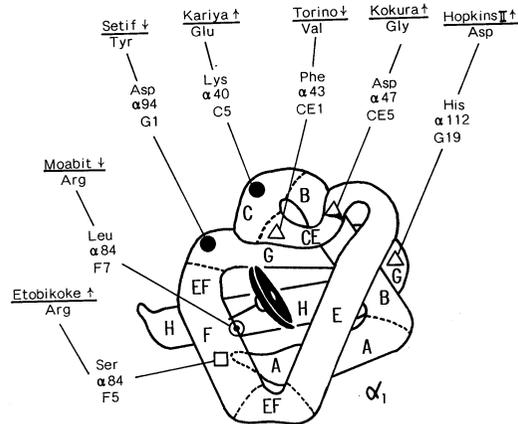 $\downarrow$ =Decreased oxygen affinity; All others have an increased oxygen affinity.

Fig. 3. α-chain variants of unstable hemoglobins with altered
oxygen affinity.
A, B, C, ..., H: helices.　　　　　CE, EF, FG: non-helices.
HC: C- or Carboxy terminus of α chain.

● $\alpha_1\beta_2$ contact　　　⊕ $\alpha_1\beta_1$ contact　　　⊙ Heme contact
□ Internal　　　△ External　　　↑ high oxygen affinity
↓ low oxygen affinity

unstable, also have an altered oxygen affinity.[101,102]　Some of these variants lead
to hemolytic anemia and show abnormalities of oxygen binding and predominant
clinical manifestations are due to accelerated red cell destruction.　The oxygen
affinity appears to be important determinant of hemoglobin concentration in
patient with congenital Heinz Bodies hemolytic anemia (CHBHA) due to an
unstable Hb, than shortening of red cell survival, as a clear inverse relationship
has been shown between oxygen affinity and PCV[103] which is based on hypothesis
that the higher the oxygen affinity, the greater the erythropoietin drive to the
bone marrow.　This has been demonstrated in many cases, e.g. patients with
Hb Köln exhibit significantly increased oxygen affinity and the resultant tissue
hypoxia stimulates erythropoiesis and these individuals have minimal anemia,
despite a significant amount of hemolysis.

CLINICAL AND LABORATORY MANIFESTATIONS

The hemoglobin variants with an altered oxygen affinity are usually inherited
as autosimal dominant but there are many examples where spontaneous mutation
has taken place and other family members of the carrier remain unaffected.
Several hemoglobins with change in oxygen affinity have an altered electro-
phoretic mobility which is demonstrable on cellulose acetate membrane at pH 8.6
and this procedure therefore, can be adopted as routine for screening.　However,
failure to demonstrate an abnormal hemoglobin by this technique does not
exclude its presence, as there are nearly one third mutants with high oxygen
affinity which are electrophoretically silent e.g. Hbs Milledgeville, Brigham,
Olympia, Vanderbilt, Bun Bury, San Diego, Syracuse and many that cannot
be separated from Hb A by electrophoresis.　In these, the application of other
methods such as citrate agar gel electrophoresis (pH 6.0) and isoelectric focusing
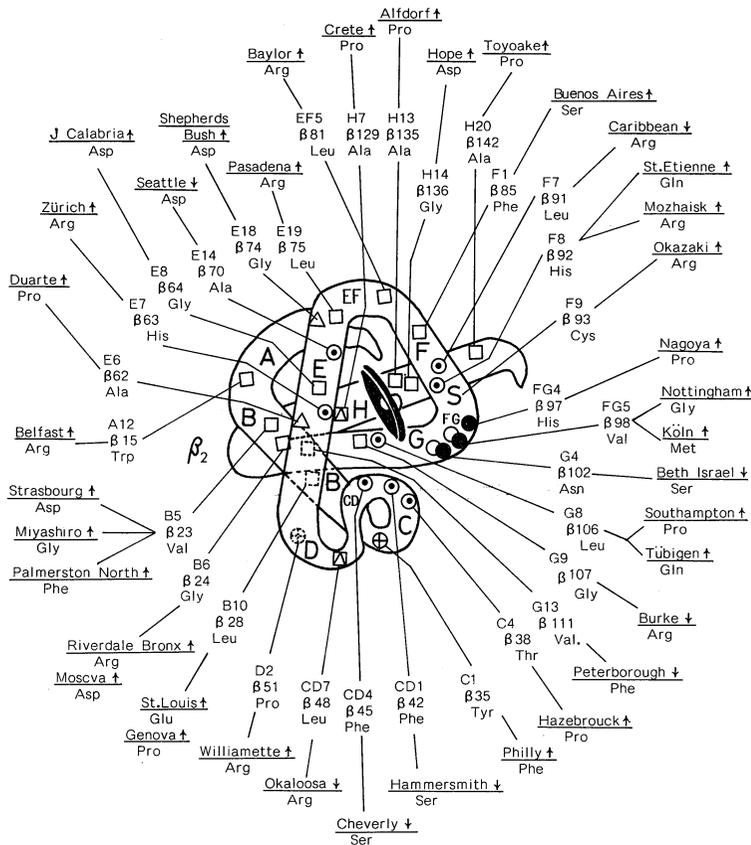
Alfdorf↑
Crete↑   Pro
Baylor↑   Pro   Hope↑   Toyoake↑
Arg   Asp   Pro
                                          Buenos Aires↑
            Shepherds   EF5 H7 H13   Ser
J Calabria↑ Bush↑   β81 β129 β135   H20   Caribbean↓
   Asp   Asp   Leu Ala Ala   β142   Arg
          Pasadena↑   Ala   St.Etienne↑
   Seattle↓   Arg   H14   F1   F7   Gln
Zürich↑ Asp   β136 β85 β91   Mozhaisk↑
   Arg   E18 E19   Gly Phe Leu   Arg
          E14 β74 β75   F8   Okazaki↑
      E8   β70 Gly Leu   β92   Arg
Duarte↑ β64 Ala   His
  Pro   E7   Gly   F9
       β63   β93   Nagoya↑
       His   Cys   Pro
   E6   Nottingham↑
   β62   FG4 FG5   Gly
   Ala   β97 β98   Köln↑
Belfast↑ A12   His Val   Met
  Arg β15   G4   Beth Israel↓
      Trp   β₂   β102   Ser
                                   Asn
Strasbourg↑   G8   Southampton↑
  Asp   B5   β106   Pro
Miyashiro↑ β23   Leu   Tübigen↑
  Gly   Val   G9   Gln
Palmerston North↑ B6   β107
  Phe   β24   Gly
         Gly   G13
      B10   C4   β111   Burke↓
      β28   β38 Val.   Arg
Riverdale Bronx↑ Leu   Thr   Peterborough↓
  Arg   Phe
Moscva↑   D2   Hazebrouck↑
  Asp   β51 CD7 CD4 CD1   C1   Pro
St.Louis↑ Pro β48 β45 β42 β35   Philly↑
  Glu   Leu Phe Phe Tyr   Phe
Genova↑
  Pro   Williamette↑
        Arg   Okaloosa↓   Hammersmith↓
              Arg   Ser
              Cheverly↓
                Ser

Fig. 4. β-chain variants of unstable hemoglobins with altered
oxygen affinity.
A, B, C, ..., H: helices.   CD, EF, FG, GH: non-helices.
NA: N- or amino terminus of β chain
HC: C- or carboxy terminus of β chain

● α₁β₂ contact   ⊕ α₁β₁ contact   ⊙ Heme contact
□ Internal   △ External   ↑ high oxygen affinity
↓ low oxygen affinity   D (HC2) deoxyl
S Active SH radical

can be very helpful in revealing the abnormality. Oxygen dissociation studies which are usually available with referal centers are essential for definitive diagnosis and can reveal whether the patient has increased or decreased oxygen affinity variant. Heat stability test is also useful since many mutants are heat labile. The abnormal hemoglobin with altered oxygen affinity with the exception of Hb Vancouver (90%) and Hb Abruzzo (94%) usually constitute about 10–50% of the total hemoglobin and the structural studies are needed to determine molecular abnormality.

Majority of the affected persons are entirely asymptomatic and the physical examination is not remarkable except for a ruddy complexion and deeply injected conjunctivae. In affected patients, an increased hemoglobin level (16–24 g/dl), a raised packed cell volume (PCV) and high red cell count above the upper normal limits in the absence of leukocytosis, thrombocytosis and splenomegaly

should suggest the presence of a hemoglobin variant with an increased oxygen affinity. An increased red cell mass without leukocytosis and thrombocytosis is usually found in hemoglobinopathies with increased oxygen affinity and mild cyanosis in those hemoglobinopathies which are associated with low oxygen affinity. The erythrocytosis due to an increased oxygen affinity is benign because the clinical syndrome has usually been found in healthy subjects and no treatment is indicated. Minor clinical manifestations of headache, lethargy occasional dizziness and easy fatigability however have also been reported in children.[104] In elperly patients, excercise intolerance, dyspnea on exertion and mild angina pectoris have been reported. If no abnormality of Hb is detectable, another possibility is decrease in the levels of 2,3 DPG perhaps due to specific enzyme deficiencies such as hexokinase deficiency and 2,3 DPG mutase deficiency.

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