

Hemoglobin Andrew-Minneapolis $\alpha_2^A \beta_2^{144 \text{Lys} \rightarrow \text{Asn}}$: A New High-Oxygen-Affinity Mutant Human Hemoglobin

By Solomon J. Zak, Bernadine Brimhall, Richard T. Jones, and Manuel E. Kaplan

The functional properties and primary structure of a new β -chain mutant of human hemoglobin are described. The mutant was transmitted as an autosomal dominant characteristic. Affected members of the kindred exhibited marked erythrocytosis due to the high oxygen affinity of the resultant hemoglobin. The abnormality is associated with a substitution of an asparaginyl residue for lysine

in the 144 position of the β -chain, $\alpha_2^A \beta_2^{144 \text{Lys} \rightarrow \text{Asn}}$, presumably due to an AAA/G to AAA/U transversion. The mutant hemoglobin displayed a profound increase in oxygen affinity, with a P_{50} of the fresh whole blood of 14 mm Hg. The isolated mutant hemoglobin exhibited near normal heme-heme interaction, a half-normal Bohr effect, and normal reactivity with 2,3-diphosphoglycerate.

AN INCREASING number of human hemoglobin variants have been reported wherein gross alterations in oxygen binding can be related to the primary structural changes.¹⁻⁸

The present report deals with a previously undescribed mutation in the β -chain ($\alpha_2^A \beta_2^{144 \text{Lys} \rightarrow \text{Asn}}$), producing a high affinity for oxygen, with resulting asymptomatic erythrocytosis as the sole clinical manifestation. This hemoglobin variant has been named hemoglobin Andrew-Minneapolis.⁹ The clinical phenotype of erythrocytosis associated with this variant is transmitted as an autosomal dominant trait.

MATERIALS AND METHODS

Routine hematologic studies were performed by standard methods. Blood specimens for hemolysates were collected in heparin for structural studies. Specimens for whole blood oxygen-hemoglobin dissociation curves were collected in heparin-sodium fluoride (10 mg NaF/ml solution containing 1000 U Na heparin). Hemoglobin solutions were analyzed by electrophoresis on cellulose acetate and starch gel, and by isoelectric focusing in polyacrylamide gel as described by Drysdale, Righetti, and Bunn.¹⁰ Whole blood oxygen hemoglobin dissociation curves were performed both by the mixing method of Edwards and Martin,¹¹ and by automatic continuous recording in a Radiometer DCA1 Dissociation Curve Analyzer.¹²

Similar studies were performed on purified hemoglobin fractions in Bis-Tris buffers at varying pH, in the presence and absence of 2,3-DPG using CO₂-free gases, at hemoglobin concentrations of 50 mg/ml. Hemolysates were fractionated by DEAE Sephadex A-50M chromatography in 5 × 30-cm columns.¹³

For primary structural studies, aminoethylated α - and β -chains were obtained as described by Clegg, Naughton, and Weatherall.¹⁴ Tryptic digests of aminoethylated β -chains were chromato-

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graphed on Aminex A-5 columns as described by Jones.¹⁵ Aliquots of the Aminex A-5 column chromatographic eluates were hydrolyzed with constant boiling HCl in vacuo at 110°C for 20 hr, brought to dryness, and applied to a Beckman/Spinco Model 120C Amino Acid Analyzer. Purified aberrant peptide was isolated and analyzed also with aminopeptidase M.

Para-mercuribenzoate derivatives were prepared as described by Rosemeyer and Heuhns.¹⁶

RESULTS

The propositus, a 37-yr-old married veteran, was noted to have a high hemoglobin and hematocrit during routine evaluation. At least two of his brothers were known to have erythremia of unknown cause. Physical examination revealed an overweight male 183 cm tall, weighing 101.5 kg. The only abnormal physical findings were plethora and a nonspecific conjunctivitis. The spleen and liver were not enlarged. The neurologic examination was within normal limits.

The hemoglobin was 19.8 g/100 ml, and packed cell volume was 57%; the white cell count was 8000/cu mm, with 58% neutrophils, 41% lymphocytes, and 1% monocytes. The red cell morphology was entirely normal. The platelets numbered 165,000/cu mm, and the reticulocyte count varied from 4.1% to 1.5% of 5.89–5.92 million red cells. The Cr⁵¹ red cell mass was 34 ml/kg (49.3 ml/kg ideal body weight), and a red cell survival study revealed a $t_{1/2}$ of 29 days. A whole blood oxygen-hemoglobin dissociation curve revealed a profound left shift as compared with the normal (Fig. 1). Similar studies with fresh whole blood of family members disclosed the pedigree described in Fig. 2.

Electrophoresis on cellulose acetate revealed two major hemoglobin components. The abnormal component migrated anodally to hemoglobin A at pH 8.2, and as shown in Fig. 3, had an isoelectric point similar to that of hemoglobin A₃. Para-hydroxymercuribenzoate treatment of the aberrant hemoglobin, previously isolated from DEAE-Sephadex, revealed an α -chain derivative which migrated identically to isolated normal α -chain derivative. In

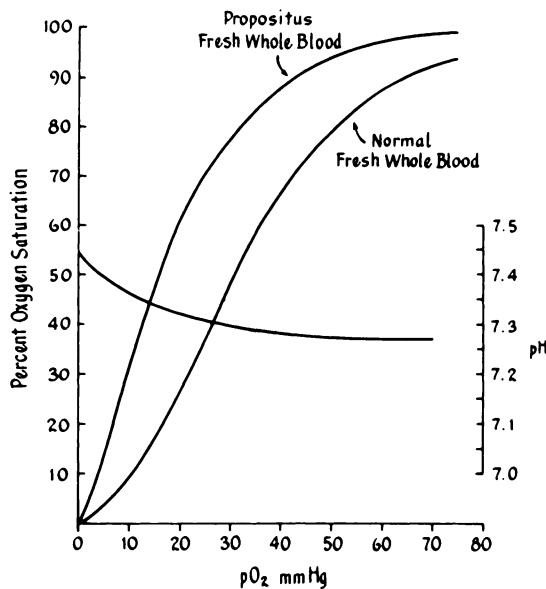


Fig. 1. Whole blood oxygen-hemoglobin dissociation curves of normal fresh whole blood compared with that of fresh whole blood of Hb Andrew-Minneapolis propositus. The curve of the normal blood is corrected for the progressive changes in pH during oxygenation, whereas the curve for the propositus' whole blood is that actually obtained.

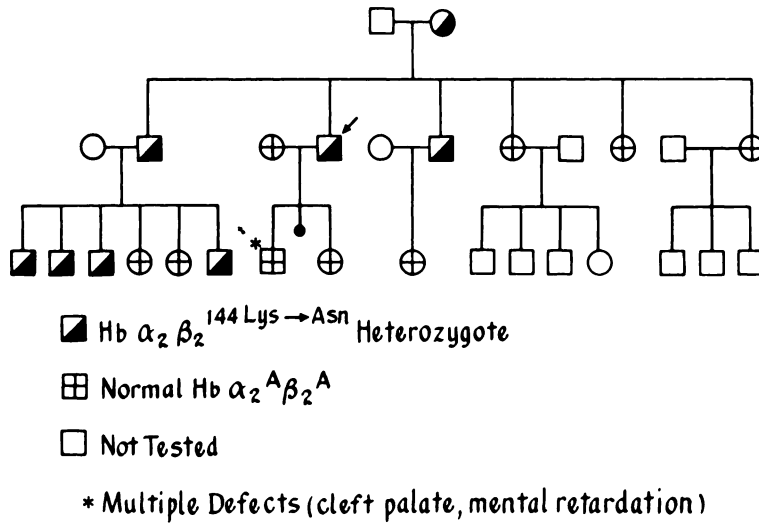


Fig. 2. Pedigree of three generations for Hb Andrew-Minneapolis $\alpha_2 \beta_2^{144 \text{ Lys} \rightarrow \text{Asn}}$. Only one still birth is noted in 23 total pregnancies. Propositus is indicated by arrow.

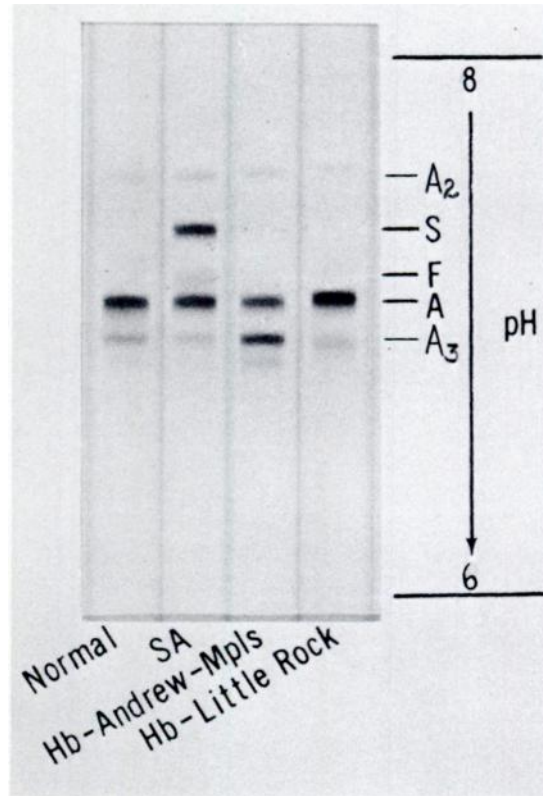


Fig. 3. Isoelectric focus pattern of normal adult red cell lysate, SA, lysate of Hb Andrew-Minneapolis, and of Hb Little Rock, in polyacrylamide gel, pH 6-8 at 2°C, after 4 hr. The photographs are those of unstained gels taken immediately at the end of the study. The faint minor band closest to pH 6 is A₂; the minor band anodal to Hb A is A₃. Note that the position of hemoglobin Andrew-Minneapolis is nearly identical to that of Hb A₃.

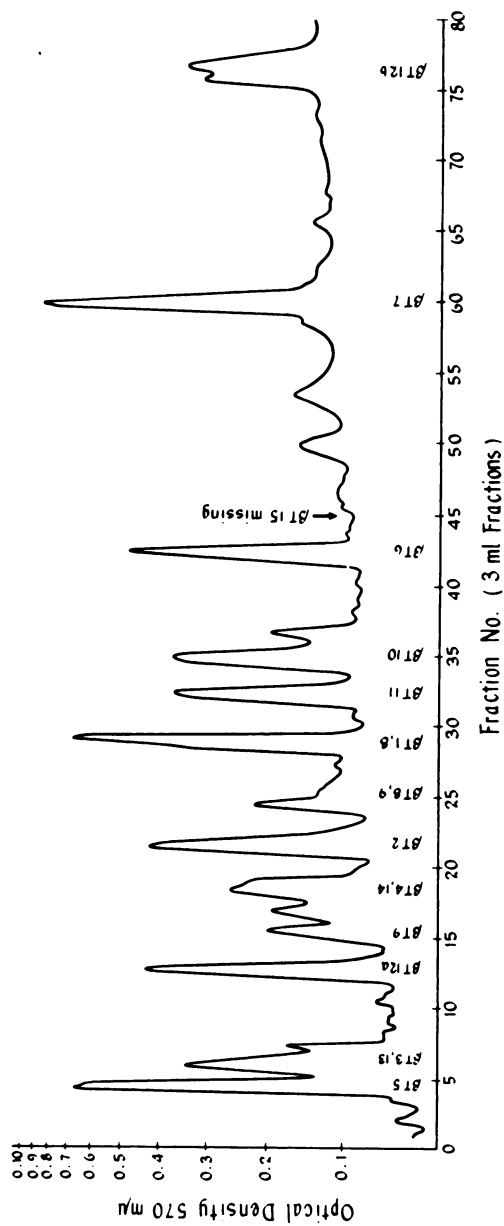


Fig. 4. Aminex A-5 Chromatogram of tryptic digest of aberrant amino-ethylated beta chain of Hb Andrew-Minneapolis, 50°C, 38 mg of tryptic linear gradient was used to develop this pattern, from 0.2 M pyridine-acetate digest applied to column equipped with a stream splitter routing 10% of pH 3.1 to 2.0 M pyridine-acetate pH 5.0. Note complete absence of β T-15 peptide, whose location is designated by arrow.

Table 1. Hemoglobin Andrew-Minneapolis $\alpha_2\beta_2^{144\text{Lys}\rightarrow\text{Asn}}$

Amino Acid Composition		β T14-15 Peptide Theoretical
Residue	Found	
Lys	0	1
His	1.95	2
Asn	2.06	1
Gly	1.02	1
Ala	4.05	4
Val	2.75	3
Leu	1.14	1
Tyr	1.09	1

Val- Val- Ala- Gly- Val- Ala- Asn- Ala- Leu- Ala- His- Asn- Tyr- His
133-134-135-136-137-138-139-140-141-142-143-144-145-146

Val- Val- Ala- Gly- Val- Ala- Asn- Als- Leu- Als- His- Lys

The new peptide
Normal β T-14 peptide
Tyr-His Normal β T-15 peptide

Amino acid composition of aberrant peptide isolated from rechromatography of T-4, 14 peak of Fig. 4 after digestion with aminopeptidase-M, demonstrating actual residue ratios compared with theoretical ratios. In the normal β -chain the Lys 144 permits tryptic cleavage to yield the terminal dipeptide. Substitution of the asparaginyl residue in place of lysine prevents tryptic cleavage.

contrast, the PMB β -chain derivative migrated anodally to the normal PMB- β -chain derivative.

The abnormal aminoethylated β -chain was readily isolated on carboxymethylcellulose columns in 8 M urea. After tryptic digestion of the abnormal β -chain, peptide chromatography on Aminex A-5 at 50°C revealed total absence of the β T-15 peptide peak (Fig. 4). Rechromatography and amino acid analysis of each peak disclosed an abnormal peptide contained in the fraction designated β T-4,14. The amino acid composition of this peptide is given in Table 1. All amino acids of the normal β T-14 peptide are present except for lysine; there is an extra asparaginyl residue, to which the normal β T-15 dipeptide, tyrosyl-histidine is linked. The substitution of asparagine for lysine is consistent with a one-step transversion from AAA/G to AAC/U.

Functional studies of the isolated mutant hemoglobin revealed a Bohr effect of -0.305 (normal using CO_2 -free gases for isolated Hb A in this laboratory is -0.50); a Hill plot disclosed a slope or n , of 2.4 (Hb A in this laboratory is 2.5–2.7). The P_{50} of hemoglobin A stripped of 2,3-DPG (by gel filtration through G-25 Sephadex at pH 7.5 at 2°C) was found to be 18.5 mm Hg, at 37°C, pH 6.95, whereas that of the variant hemoglobin under identical conditions, was 3.3 mm Hg. The addition of 2,3-DPG in stoichiometric amounts shifted the P_{50} of hemoglobin Andrew-Minneapolis to the right from 3.3 to 6.0 mm Hg, at pH 6.95, 37°C; the rightward shift for hemoglobin A under identical conditions was from 18.5 to 34.5 mm Hg, a similar percentage shift.

DISCUSSION

According to the allosteric model of human deoxyhemoglobin as derived by the Cambridge group,^{18,19} the rotational freedom of the HC terminal residues of all four globin chains is restricted by salt bridges. In the β -chain, the α -carboxyl of histidine HC3 (146) β_1 is linked to the ϵ amino group of lysine C5(40) α_2 , and the imidazole of HC3 is linked to aspartate FG1(94) β_1 . Each penultimate

tyrosine of the β -chains is so situated in the FG interhelical segment that its displacement results in disruption of these C-terminal salt bridges. It follows that if movement of the phenolic side chain of tyrosine HC2(145) were restricted so that it could not readily enter the interhelical FG segment, initiation of the bond ruptures leading to the oxy-configuration would be favored. This would leave intact the polyfunctional role of the C-terminal histidine, resulting in a normal or near normal heme-heme interaction, upon oxygenation or deoxygenation. However, the oxygenation process itself would proceed with the molecule energetically predisposed to the oxy-configuration, and thereby exhibit high affinity for oxygen. In accord with the observations of Perutz,¹⁷ Hayashi and Stamatoyannopoulos have recently reiterated the importance of tyrosine HC2 in relationship to the increased oxygen affinity of hemoglobins Rainier $\alpha_2^A\beta_2^{145\text{Tyr}\rightarrow\text{Cys}}$, and Bethesda $\alpha_2^A\beta_2^{145\text{Tyr}\rightarrow\text{His}}$.²⁰ However, three other high-affinity stable human hemoglobin variants involving residues close to the carboxyterminus do not directly involve HC2. These are hemoglobins Little Rock $\alpha_2^A\beta_2^{143\text{His}\rightarrow\text{Asn}}$, Hiroshima $\alpha_2^A\beta_2^{146\text{His}\rightarrow\text{Asp}}$, and Andrew-Minneapolis $\alpha_2^A\beta_2^{144\text{Lys}\rightarrow\text{Asn}}$. It is apparent that all of these high-affinity mutants involve substitutions more acidic than the residue usually present in the β -chain. It is postulated that in these mutants, the HC segment assumes helicity resulting in relative limitation of HC2 rotational motion, but not total exclusion from the interhelical FG segment in the de-oxy state, wherein it forms its stabilizing bond with the carbonyl of valine FG5. This helicity does not, however, affect the polyfunctional hydrogen bonding of HC3 as described above, providing insight into the normal or near normal heme-heme interactions reported for these three mutants. In hemoglobin Andrew-Minneapolis, with the substitution of asparagine for lysine CH1(144) β , it is postulated that a hydrogen bond is formed between the amide oxygen of asparagine HC1 and the imidazole of the C-terminal histidine HC3(146) β , resulting in restricted movement of the phenolic side chain of the penultimate tyrosine residue (Fig. 5). The mechanism postulated for such a restriction would be extension of the H helix to include the terminal three amino acids. Furthermore, the binding of the imidazole of HC3 by the amide oxygen of asparagine 144 makes the former unavailable for its contribution to the Bohr effect, resulting in the half-Bohr effect found experimentally. Finally, since all seven coordination sites for binding of 2,3-DPG remain intact and available,²¹ interaction of the mutant with 2,3-DPG is predictably normal.

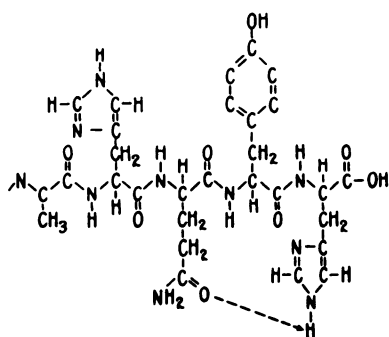


Fig. 5. Schematic of the terminal five residues of aberrant β -chain of Hb $\alpha_2^A\beta_2^{144\text{Lys}\rightarrow\text{Asn}}$ showing the proposed site of hydrogen bonding between the amide oxygen of the asparaginyl residue and the imidazole proton of the terminal histidine.

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