ABNORMAL FUNCTIONAL PROPERTIES OF Hb HOPE $\alpha C_2^A \beta_2$ (H14) GLY → ASP: A LOW OXYGEN AFFINITY HEMOGLOBIN WITH DECREASED DPG EFFECT

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1. Introduction

Hb Hope β136 (H14) Gly → Asp was found initially by Minnich et al. in 1965 [1] in an American Negro family. He published the structure and described Hb Hope as a clinically silent hemoglobin. The function was not studied. Perutz in 1968 [2] proposed as a hypothesis, the formation of a new salt bridge between Asp 136 and Val N_1 of the Hb Hope β chain. This paper describes the functional properties of a new case of Hb Hope found in a propositus native of the Mali Republic. This study shows a decreased oxygen affinity, a decreased cooperativity, a normal Bohr effect and a decreased DPG effect. Molecular interpretation of these abnormalities is discussed and Hb Hope properties are compared with those of others hemoglobins presenting a modified DPG effect.

2. Materials and methods

The propositus was a 25 year old Negro native of Mali, hospitalized after an episod of articular inflammation of his knee followed by abdominal pains. Hematologic studies were performed according to standard methods.

2.1. *Identification of the mutation*

The abnormal hemoglobin was isolated by column chromatography on amberlite Biorex 70 in a 0,04 M phosphate buffer pH 7.12 [3] without cyanide. Localization of the abnormal peptide was obtained by fingerprints on silica gel [4]. Structural analysis of the abnormal peptide was performed after its isolation and purification on ion exchange column chromatography. [5].

2.2. *Determination of functional properties*

Oxygen equilibrium curves were determined on red blood cells and on hemoglobin solutions by the spectrophotometric method of Benesch [6]. Oxygen equilibria of isolated Hb A and Hb Hope were determined using hemoglobins stripped by chromatography on Sephadex G 25 [7]. Equilibrium curves of the red blood cells were measured at 37°C in 0.15 M phosphate buffers at pH ranging from 6.45 to 7.45; the curves of stripped hemoglobins were measured in 0.05 M bis–Tris or 0.05 M Tris buffers according to the pH values. P_{50} of Hb A and Hb Hope were determined in presence of varying amounts of 2,3-diphosphoglycerate (DPG) from Benesch's technique [8] modified using 0.05 M Tris buffer at pH 7.15. Increasing amounts of DPG were introduced into the tonometer before each determination. The cyclohexylammonium salt of DPG (Calbiochem) was used after conversion to the free acid form [9] and titration to neutrality. Amounts of methe moglobin were measured before and after each determination according to Benesch [10].

The binding of DPG to hemoglobin was measured with an ultrafiltration technique using cellulose nitrate membranes in a high pressure apparatus [11]. The binding experiments were carried out at pH 7.2 in bis-Tris buffer 50 mM 0.1 M Cl^-, on deoxyhemoglobin at a concentration of free DPG of approximately 5×10^{-5} M. Hemoglobin concentration was 5×10^{-5} M on a tetramer basis, temperature was 22°C. The DPG concentrations in the Hb–DPG mixture and in the ultrafiltrate were determined by the enzymatic method of Rose [12].

3. Results

Clinical and hematologic findings demonstrated only the presence of a slight anemia. The Coultronic S furnished the following values: Hb 12–13 g%, RBC 4.3×10^6, PCV: 36.5, MCV: 86 μ^3, MCH: 28, MCHC 33%. Reticulocyte count was 55 000, WBC count was 6000/mm^3. Total blood volume was 3400 ml (normal: 3500 ml), packed red cell volume: 1180 ml (normal: 1400), plasmatic volume: 2200 ml (normal: 2000). This anemia was isolated, without hemolytic symptoms, enlargement of the spleen, or anti red cells antibodies. This anemia with a decreased red cell mass is probably due to a slight decrease of the bone marrow activity.

Electrophoresis of hemoglobin on cellulose acetate strips at pH 8.6 demonstrated the presence of an abnormal hemoglobin representing approximately 40% of the total

3.1. *Structural studies*

Results of (i) electrophoresis of hemoglobin and its chains, (ii) fingerprints of the S-aminoethylated β chain were identical to those obtained by Minnich. We have used a distinct method for isolating abnormal peptide. Elution profil of chromatography of the tryptic hydrolysate S-AE β chain is presented in fig. 1 which shows the position of the mutated peptide where amino acid analysis demonstrated replacement of the 136 Gly by an aspartic acid.

3.2. *Oxygen equilibrium curves*

Results of oxygen equilibrium curves of the intact red blood cells from the patient are presented in fig. 2. At the pH tested, the patient's curves are shifted to the right of the normal curves at the corresponding pH. P_{50} (partial pressure of oxygen at 50% Hb O_2) at pH 6.45, 7.15 and 7.45 were 62, 42 and 31 mmHg

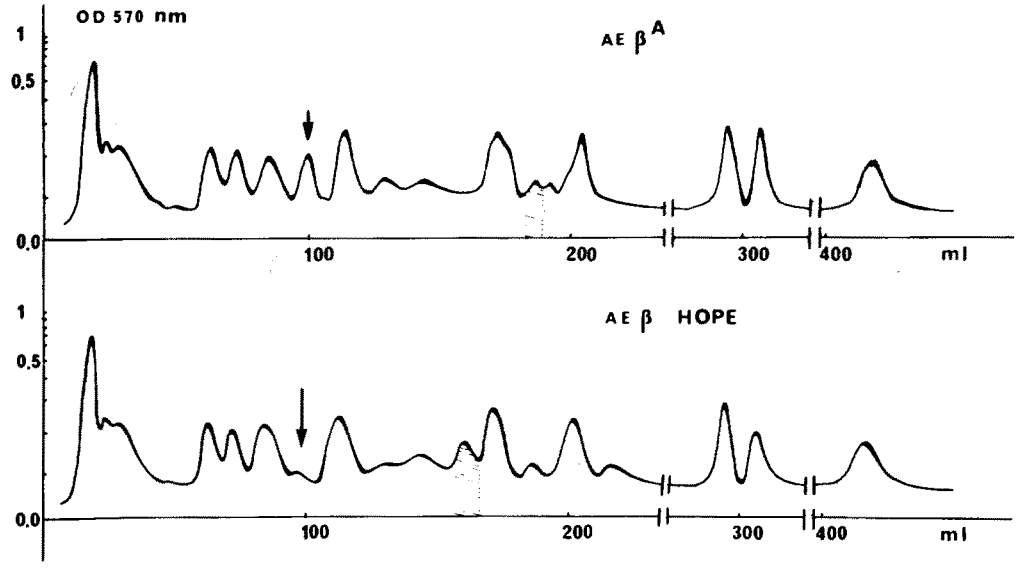


Fig. 1. Elution profiles of chromatography of the tryptic digest of normal and abnormal aminoethylated β chains on PA 35 (Beckman) exchange ion resin. From top to bottom chains β^A and β^{Hope}. Hatched zones indicate the positions of the βT14-15 peptides, the arrows show the normal position of the βT14 peptide.

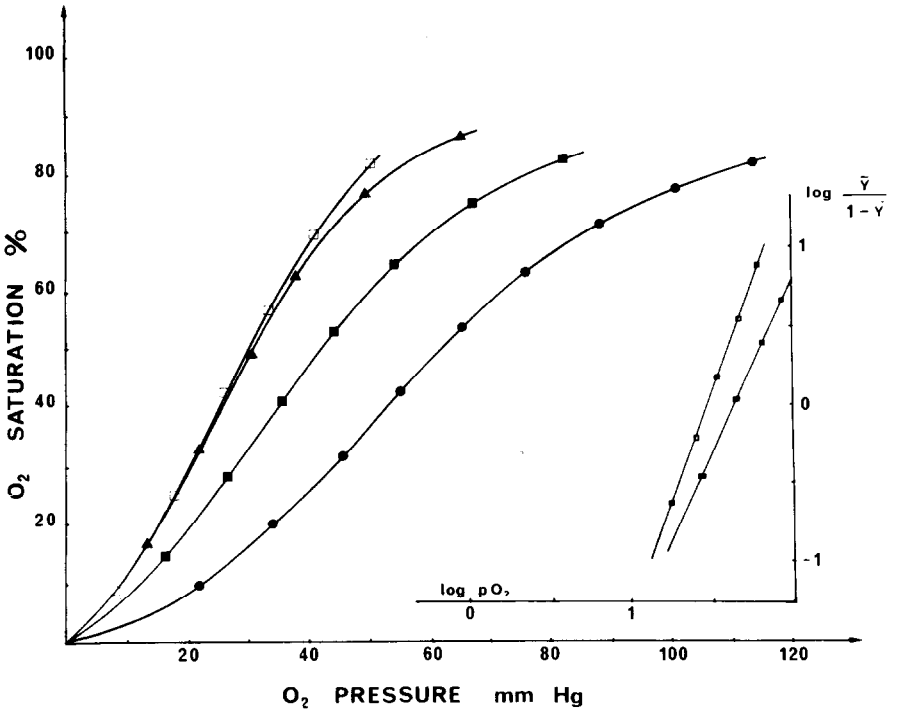
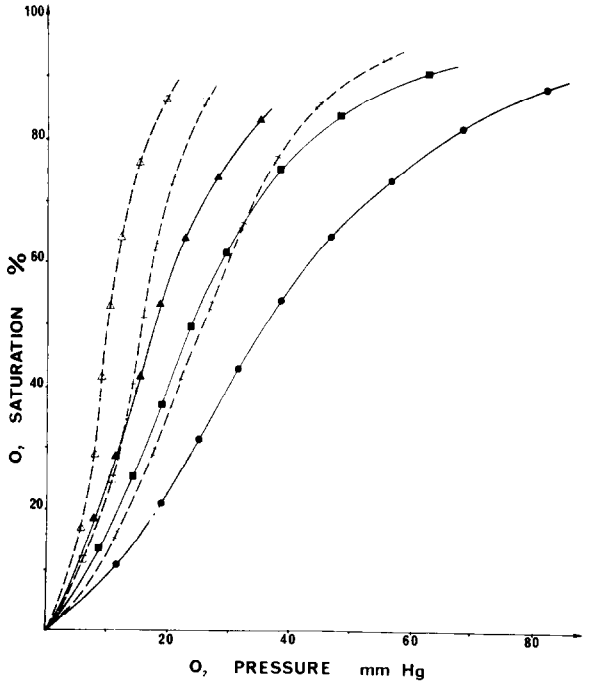


Fig. 2. Oxygen dissociation curves determined at 37°C on red cells suspension from the patient containing 60% of Hb A and 40% of Hb Hope at pH 6.45 (——●——●——), pH 7.15 (——■——■——) and pH 7.45 (——▲——▲——▲) and from control normal red cells at pH 7.15 (——□——□——). On the right side of the figure Hill's plot of the data obtained at pH 7.15 from the control (——□——□——) and from the patient red cells (——■——■——).



respectively (normal values 52 ± 2, 30 ± 1 and 22 ± 1 mmHg). These shifts could not be explained by the normal range of the DPG level : 15 μM/g Hb. Hill plot representation produced no evidence of biphasic curve.

The results of the oxygen equilibrium curves of isolated Hb Hope and Hb A are represented in fig. 3. They show that Hb Hope has a lowered oxygen affinity, with a preserved alkaline Bohr effect (fig. 4). The Hill's coefficient was found to be 2.2 (normal 2.8), indicating a decreased cooperativity.

Fig. 3. Oxygen equilibria curves of purified and stripped hemoglobin A and hemoglobin Hope. (——△——△——△——), Hb A at pH 7.45; (——□——□——□——), Hb A at pH 7.15; (——○——○——○——), Hb A at pH 6.45; (——▲——▲——▲——), Hb Hope at pH 7.45; (——■——■——■——), Hb Hope at pH 7.15; (——●——●——●——), Hb Hope at pH 6.45.

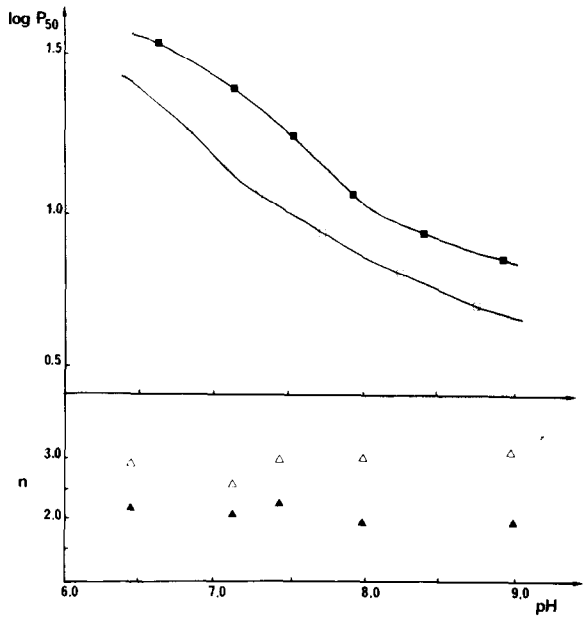


Fig. 4. The pH dependence of the oxygen equilibrium of hemoglobins Hope (——■——■——) and Hb A (——□——□——). The *'n'* range value (——▲——▲——) is (2.3–2.0) for Hb Hope and (2.6–3.1) for Hb A (——△——△——).

3.3. *Effect of DPG on Hb Hope*

The P_{50} of stripped Hb A and Hb Hope were then measured after addition of increasing amounts of DPG. The amounts of methemoglobin A and Hope were found to be 1.5% at the beginning of these experiments and never exceeded 3.5% at the end of them. Results of variations of P_{50} according to the level of DPG are represented in fig. 5. In our findings the maximum effect of DPG added to Hb A was obtained with a DPG/Hb A molar ratio of 20, the displacement of P_{50} being of 27 mmHg. In contrast amounts of DPG required for giving the plateau with Hb Hope were twice those

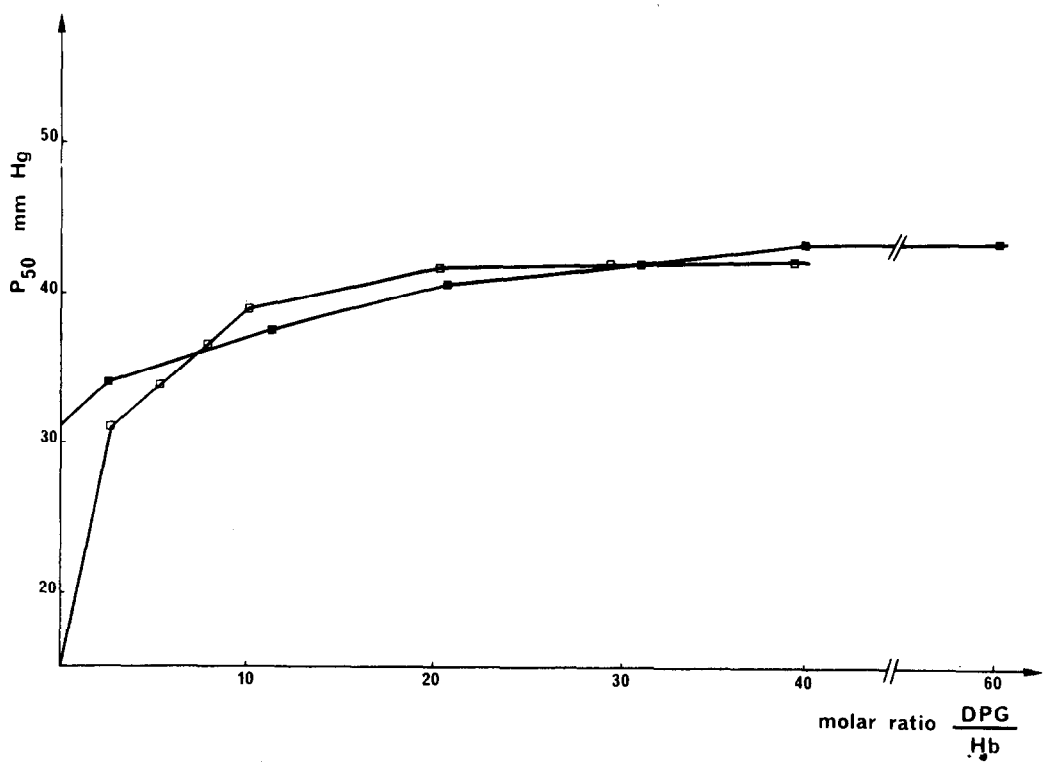


Fig. 5. Action of increasing amounts of DPG on P_{50} of Hb Hope (——■——■——) and Hb A (——□——□——). The P_{50} obtained at 37°C in Tris–HCl 0.05 M pH 7.15 are plotted as a function of the $\frac{DPG}{Hb}$ ratio.

necessitated for Hb A. These results indicate that the reaction of Hb Hope with DPG is abnormal. To find the causes of this abnormal reaction we have measured the DPG/deoxyhemoglobin Hope dissociation constant. This was done by a direct method using the ultrafiltration technic. The apparent dissociation constant K' was found to be 12×10^{-5} M for Hb Hope. In the same experimental conditions K' was found to be 1.5×10^{-5} M for normal Hb A.

Thus the abnormal reaction of DPG with Hb Hope is mostly due to a low affinity of this ligand for Hb Hope.

4. Discussion

Hb Hope is a new case of hemoglobin with a decreased oxygen affinity. This functional modification is found in the absence of methemoglobinisation as in Hb M Iwate [13] or M Boston [14] and also, in the absence of instability as in Hb Seattle [15,16], Hammersmith [17], Torino [18], Bristol [19], Tacoma [20], Bucuresti [21] and Okaloosa [22].

Numerous cases of abnormal hemoglobins with high oxygen affinity have been described but only 5 cases of abnormal hemoglobins with isolated low O_2 affinity are known: Agenogi [23], Kansas [24], Yoshizuka [25], Peterborough [26] and Hope. These hemoglobins lead to a decreased red blood cell volume. The mechanisms implicated may concern the degree of stimulation of synthesis of erythropoietic [27].

To explain the structure function relationship of these low O_2 affinity hemoglobins, two major distinct types of mechanisms have been evocated [28]: (i) the mutation modifies the conformation of the heme pocket, thus inhibiting the entrance of the O_2 molecule: this is the case, for example, of Hb Hammersmith and of Hb Bristol; (ii) the substitution favorizes the deoxystate by a purely stereochemical effect as in the case of Hb Peterborough β (G 13) Val → Phe where the substitution is included in the $\alpha_1\beta_1$ contact area and where the new Phe can be accomodated only in the deoxy form.

An other example is Hb Agenogi β (F6) Glu → Lys where the lysine probably interacts with the C terminal residue of the β chain, maintaining the HC2 tyrosine in its pocket.

The mechanism hypothesized for Hb Hope is different. We assume that the salt bridge binding Asp β H14 with the α amino group of Val β NA_1 can maintain the β chain in a tight conformation 't' which displaces towards the left the deoxy ⇆ oxy equilibrium.

Absence of modification of the Bohr effect signifies that residues implicated in this effect are not involved by the mutation.

In addition, the abnormal bond between H14 and NI can also explain the abnormal reaction between Hb Hope and DPG. In Hb Hope, DPG fixation in the central cavity is partially inhibited since NA_1 which normally is involved in an ionic bond with DPG is blocked-Hb Hope is a new case of Hb with a mutation involving the central cavity and giving rise to a decreased DPG effect. In all the previously described cases: Hb Bethesda [29], Hb Sheperds Bush [30], Hb Leiden [31] [32] and Hb Little Rock [33], the low observed DPG effect was associated with a high oxygen affinity. The opposite effect is observed with Hb Hope. Interpretation of this discrepancy can afford new elements in the stereochemical mechanisms involved in the Hb ligand binding.

Moreover in this work, we have used two distinct approaches to estimate the DPG effect on Hb Hope. Results obtained by the two technics are very similar. The direct measurement of dissociation constant DPG/deoxyhemoglobin by the ultrafiltration method has been applied for the first time in this work to an abnormal hemoglobin. Its use seemed to us to be generalized because it is a very fast technic and mostly because it affords more precise information on the impairment of DPG effect.

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