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Hemoglobin Grange-Blanche [β27(B9) Ala→Val], a new variant with normal expression and increased affinity for oxygen

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Hemoglobin Grange-Blanche [β27(B9) Ala→Val] is a new variant found in a Portuguese family. The carriers present moderate erythrocytosis. Upon isoelectric focusing, Hb Grange-Blanche was slightly more cathodic than Hb A. $\beta^{\text{Grange-Blanche}}$ chain migrated like the $^{G}\gamma$ chain when submitted to electrophoresis in the presence of urea-Triton X-100. The precentage of Hb Grange-Blanche was about 50% in the heterozygous state. Oxygen affinity was increased ($P_{50}=22$ mmHg), but heme-heme interaction was normal. An abnormal tryptic peptide (βT3) was isolated using HPLC. Its composition allowed us to deduce unambiguously the amino acid change. The latter is the third mutation found in position 27 of the β-chain. Because of its normal expression and its elevated affinity for oxygen, Hb Grange-Blanche contrasts with Hb Knossos [β27(B9) Ala→Ser], a β-thalassemic variant with low affinity.

Globin chain variant; Oxygen affinity; Peptide mapping

1. INTRODUCTION

Over 70 variants of the human hemoglobin β-chain display an increased affinity for oxygen [1], the most common functional alteration caused by abnormal hemoglobins. Such variants are usually recognized because of erythrocytosis. Six mutations involving the B helix (position 19–34) and yielding an increased affinity have been described [1]. We report herein on Hb Grange-Blanche [β27(B9) Ala→Val], a new variant in this region with an elevation of oxygen affinity and a normal heme-heme interaction.

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Abbreviations: Hb, hemoglobin; β^{GB}, $\beta^{\text{Grange-Blanche}}$; IEF, isoelectric focusing; PAGE, polyacrylamide gel electrophoresis

2. CASE REPORT

The propositus was born in Silves, Portugal, in 1954. She had no medical record until last year when she started complaining of abdominal pains. Echography showed no gall-stone, but revealed a spleen enlargement. The cause of the latter, however, turned out to be apparently unrelated to the abnormal hemoglobin. The red cell indices indicated moderate erythrocytosis (RBC, 5.16 $T \cdot l^{-1}$; Hb, 155 $g \cdot l^{-1}$; PCV, 0.48 $l \cdot l^{-1}$). The reticulocyte

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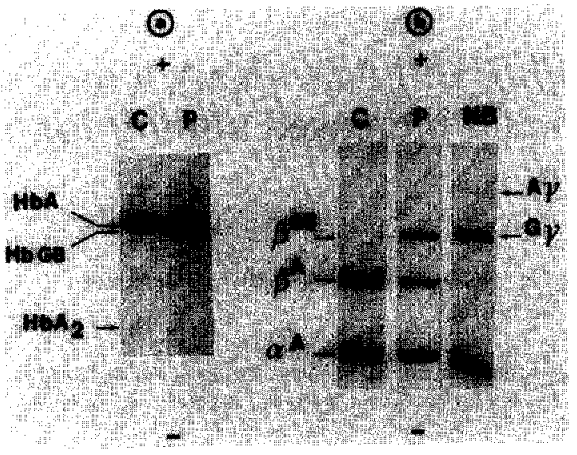

Fig.1. Electrophoretic analyses. C, control; P, propositus; NB, newborn. (a) Hemoglobin IEF on polyacrylamide gel [2]. (b) Urea-Triton PAGE of globin chains [3].

count was 35×10^9 l^{-1} and Heinz bodies were absent. The propositus has two daughters, who were born in 1972 and 1974. The eldest is hematologically normal. The youngest has a comparable picture (RBC, 5.21 $T \cdot l^{-1}$; Hb, 15 $g \cdot l^{-1}$; PCV, 0.45 $l \cdot l^{-1}$), but no splenomegaly. In all three persons the percentages of Hb A_2 and Hb F found were in normal range.

3. MATERIALS AND METHODS

Hemoglobin screening techniques included IEF of total hemoglobins on polyacrylamide gel [2] or agarose gel (Sebia, Issy-les-Moulineaux, France), and urea-Triton-PAGE of globin chains [3]. The assay of Hb A_2 relied on chromatofocusing [4] and that of Hb F on the alkali-resistance procedure [5]. An oxygen equilibrium curve was obtained from whole blood in a Hemoscan differential spectrophotometer. Hemoglobin stability was evaluated by the isopropanol procedure [6]. 2,3-Diphosphoglycerate (2,3-DPG) was assayed according to Rose and Liebowitz [7]. Structural analysis of the variant was carried out as has been detailed or referred to elsewhere [8]. Briefly, globin chains were separated on CM-52 cellulose. Following aminoethylation and tryptic digestion, the peptides were resolved using HPLC. All peptides were submitted to acid hydrolysis and their amino acid composition determined.

4. RESULTS

IEF on polyacrylamide gel revealed a hemoglobin variant 1 mm from Hb A on its cathodic side (fig.1). The abnormal β-chain migrated as the $^{G}\gamma$ chain upon urea-Triton-PAGE (fig.1). The variant, hereafter called Hb Grange-Blanche, accounted for 50% of the sum (Hb A + Hb Grange-Blanche) (table 1).

The oxygen equilibrium curve was shifted leftward (fig.2). P_{50} was slightly decreased (table 1). For low P_{O_2} values, the Hill plots bent as in controls, failing to display a genuinely biphasic character. The red cell 2,3-DPG was also normal. Finally, normal stability was found.

Table 1

Structural and functional data concerning Hb Grange-Blanche

	Hb Grange-Blanche (%)	β^{GB} (%)	P_{50} (mmHg)	$\bar{n}$	2,3-DPG (μmol/g Hb)
Mother	50.6	54	22	2.87	15.2
Daughter 1	0	0	25	2.72	12.4
Daughter 2	49.3	n.d.	22	2.98	14.3
Day control			26.7	2.91	11.8
Controls (n = 15)			27.2 ± 1.57	2.69 ± 0.11	12.83 ± 1.90

The percentage of Hb Grange-Blanche with respect to the sum (Hb A + Hb Grange-Blanche) was determined by scanning the isoelectric focusing agarose gels (not shown) at 525 nm. The percentage of β^{GB} chain with respect to total β-chains was determined by scanning urea-Triton X-100 gels at 570 nm; $\bar{n}$, slope of the Hill plots for 30 mmHg < P_{O_2} < 50 mmHg. n.d., not determined

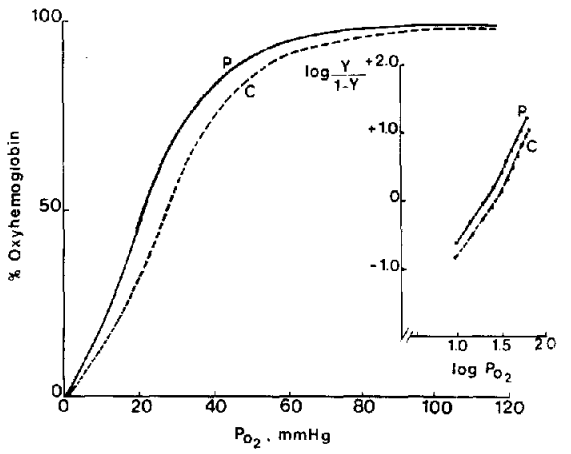

Fig.2. Oxygen equilibrium curves. C, control; P, propositus. Inset: Hill plots.

β^A and β^{GB} chains coeluted upon CM-52 ion-exchange chromatography. Following aminoethylation and tryptic digestion of mixed β-chains, HPLC analysis of the peptides revealed an additional peak (fig.3). Its amino acid composition was that of a modified βT3 peptide: Asx, 2.10 (2); Glx, 1.95 (2); Gly, 2.92 (3); Ala, 0 (1); Val, 4.02 (3); Leu, 1.14 (1); Arg, detected (1). There was no other ectopic peptide; the amino acid composition of all regular peptides was determined and found to be normal. Taken together, the present data are consistent with the Ala→Val substitution in position 27, a previously undescribed mutation as far as we are aware.

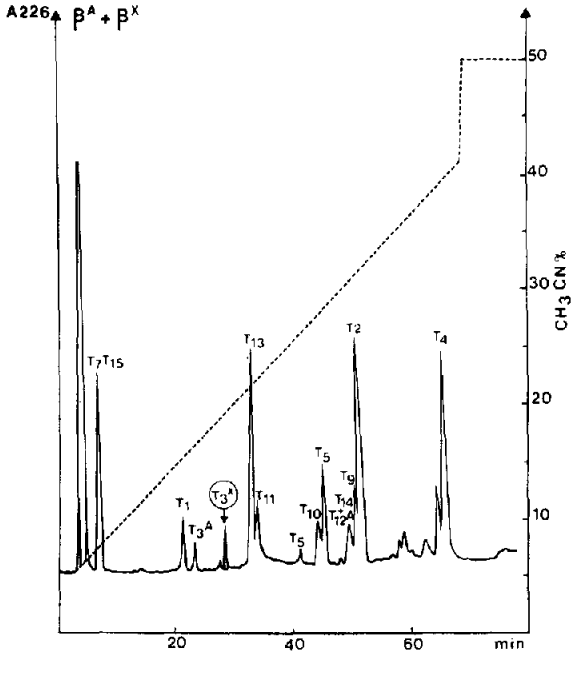

Fig.3. HPLC profile of the tryptic digest of mixed, aminoethylated normal (β^A) and abnormal (β^X) chains.

5. DISCUSSION

After Hb Volga [β27(B9) Ala→Asp] [9] and Hb Knossos [β27(B9) Ala→Ser] [10], Hb Grange-Blanche [β27(B9) Ala→Val] is the third abnormal hemoglobin found with an amino acid substitution in position 27 of the β-chain. The electrophoretic behaviour of this globin variant and its 50% level in the heterozygous state should allow rapid presumptive diagnosis in future cases.

Hb Grange-Blanche contrasts with Hb Knossos in several respects. (i) Although both hemoglobins derive from electrically neutral mutations, Hb Grange-Blanche is readily detected using isoelectric focusing, while Hb Knossos is not; the slight increase of p*I* concerning the former must reflect a conformational change. (ii) Upon urea-Triton-PAGE, the β^{GB} chain, resulting from a non-polar to a more non-polar substitution, has a slower migration toward the cathode than the β^A chain whereas the $\beta^{Knossos}$ chain, resulting from non-polar to polar amino acid substitution, has a faster migration. (iii) Hb Grange-Blanche has an increased oxygen affinity while Hb Knossos has a reduced affinity. (iv) The 50% level of Hb Grange-Blanche denotes a normal expression of the mutant β-chain. This contrasts with the depressed synthesis of $\beta^{Knossos}$ chain, which is responsible for its thalassemic character (see [8,11], for more details).

In the B helix, residue β27 occupies an internal position. It does not participate in the $\alpha_1\beta_1$ or in the $\alpha_1\beta_2$ contacts, nor is it involved in the heme pocket [12]. Although all peptides of the HPLC profile are derived from an equimolar mixture of the β^A and β^{GB} chains (unseparated by CM-52 chromatography), their normal amino acid composition makes it unlikely that the reduced P_{50} of Hb Grange-Blanche would be accounted for by a second mutation. Further studies will be necessary to establish why the β27(B9) Ala→Val substitution yields increased affinity for oxygen.

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