

# Hemoglobin Roseau-Pointe a Pitre $\alpha_2\beta_290$ (F6) Glu $\rightarrow$ Gly: a new hemoglobin variant with slight instability and low oxygen affinity

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Received 22 February 1985

A Dominican neonate carrying a new abnormal hemoglobin, hemoglobin Roseau Pointe-à-Pitre  $\alpha_2\beta_290$  (F6) Glu  $\rightarrow$  Gly, was detected in Guadeloupe during application of a cord blood screening program. This variant behaved in isoelectrofocusing as an Hb D, and displayed instability and low whole blood oxygen affinity. In the affected family it was present, either isolated, or in association with a  $\beta^+$  thalassemia trait.

*Hemoglobinopathy     $\beta$  variant-Guadeloupe     $\beta$  variant-Dominica    Hb Roseau-Pointe à Pitre  
Molecular instability    Low oxygen affinity    Structural analysis*

## 1. INTRODUCTION

A critical point in the field of human hemoglobin variants is to be able to establish rapidly whether a newly detected rare variant is undescribed, and if it induces a molecular dysfunction. During the course of a systematic cord blood screening program developed in Guadeloupe in the last 2 years, approx. 1 neonate in 1000 was found to be a carrier of a rare variant of a hemoglobin  $\beta$  gene. Most of these cases are Hb D Korle-Bu or Hb D Punjab traits. Electrophoretic data, stability studies and oxygen affinity determinations permitted us to postulate that one of these Hb D variants observed during this cord blood screening program was not previously described, at least not in Guadeloupe. Therefore, we performed a structural study which demonstrated a Glu to Gly substitution at the  $\beta 90$  (F6) residue. This variant, homologous to Hb Agenogi Glu  $\rightarrow$  Lys  $\beta 90$  (F6), a low oxygen affinity abnormal Hb [1], was named Hb Roseau-Pointe à Pitre.

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## 2. MATERIALS AND METHODS

Hematological data were obtained by standard procedures. Isoelectrofocusing (IEF) was carried out on thin-layer polyacrylamide gels in a pH 6–9 gradient [2]. The electrophoretic protocol of Schneider [3] was followed. Electrophoresis on acetate cellulose strips (Sebia, France) was performed with Tris-EDTA-borate buffer, pH 8.6. Citrate agar electrophoresis (pH 6.2) was performed with a commercial kit (Titan IV-Helena) and globin chain electrophoresis was carried out in 6 M urea, 1%  $\beta$ -mercaptoethanol (pH 6 and 9) on cellulose acetate strips (Sebia). Acid urea Triton polyacrylamide gel (PAGE) electrophoresis was performed as in [4]. Densitometry of IEF slabs and other electrophoretic profiles were obtained with a Cellosystem Sebia (France) instrument. Quantification of HbF was achieved as in [5] (normal range: 0–1%) and the HbA<sub>2</sub> level was measured with the microchromatographic procedure [6] (normal range 2.1–3.1%). Stability was checked by the isopropanol test [7]. The oxygen affinity of the whole blood was determined with an Aminco

Hemoscan (Silver Springs, USA) and the 2,3-DPG assay was performed as in [8].

Structural analysis was conducted on the aminoethylated abnormal  $\beta$  chain prepared by CM-cellulose (CM52, Whatman) chromatography [9] and desalted through a Biogel P<sub>2</sub> column (BioRad). Tryptic peptide separation of the Hb Roseau-Pointe à Pitre  $\beta$  chain was achieved by reverse phase HPLC using a microbondapak C18 column (Waters Assoc.) [10] with a precolumn and a guard column of the same phase, on a Beckman 332 apparatus equipped with a computing integrator.

### 3. RESULTS

#### 3.1. Case report

The trait was first discovered by IEF of the cord blood hemolysate of a black newborn, whose parents were of Dominican origin. A very low proportion of Hb A<sub>0</sub> was measured (~1%) compared to ~10% for the Hb D variant. The isoelectrofocusing point of this Hb D was similar to that of Hb D Korle-Bu, and thus distinctly more anodal than that of Hb S [2] as shown in fig.1a; lane 2 represents the propositus' hemolysate at 2 months of age. Six months later, the proportions of the different Hbs in the hemolysate were: Hb D, 62.5%; Hb A<sub>0</sub>, 15.1%; Hb F, 17.3%; Hb A<sub>2</sub>, 5.3%. The mother carried the trait (fig.1a) (Hb D, 36%; Hb A<sub>2</sub>, 2.2%) and the father had the typical biological features of a  $\beta$  thalassemia trait (Hb A<sub>2</sub>,

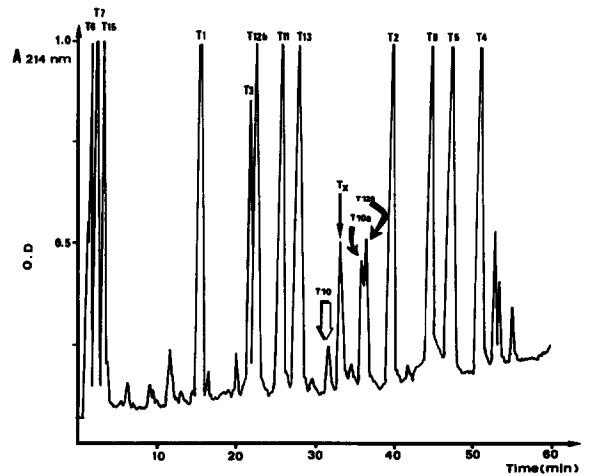


Fig.2. Elution profile of the tryptic digest of the aminoethylated  $\beta$  chain of hemoglobin Roseau-Pointe à Pitre by reverse phase HPLC. The amino composition of peptide T<sub>x</sub> is given in table 1.

5.6%; MCV, 78 fl). It was concluded that the propositus carried an association of Hb D and  $\beta^+$  thalassemia traits. Hematological data for the propositus were at the same period: RBC,  $4.54 \times 10^{12}/l$ ; Hb, 10.8 g/dl; PCV, 31.6%; MCV, 69.6 fl; MCH, 23.2 pg; reticulocytes,  $33 \times 10^9/l$ . The mother had a moderate microcytic anemia (RBC,  $4.77 \times 10^{12}/l$ ; Hb, 11.9 g/dl; PCV, 36.7%; MCV, 77 fl; MCH, 25 pg) explained by a post-partum iron deficiency. An  $\alpha$  thalassemia could not be excluded but the family was not accessible for further studies. Despite the absence of overt hemolytic anemia, biological signs of an unstable hemoglobin were obvious. There were Heinz bodies in a large number of red cells after 3 h incubation with brilliant cresyl blue and the isopropanol test was positive after 10 min (control 60 min), either with the propositus' or the mother's hemolysate.

The electrophoretic mobilities, determined as proposed by Schneider and Barwick [11] were: -4.3 for cellulose acetate electrophoresis, pH 8.7; a value clearly different from that of Hb S and of Hb Korle Bu (fig.1b); 1.3 for citrate agar electrophoresis, pH 6.2; 17.4 and 18.2 for globin chain electrophoresis urea (6 M), pH 9.0 and 6.0, respectively; and 19.4 for acid urea Triton PAGE electrophoresis. These values did not correspond to any previously described variant [13]. The

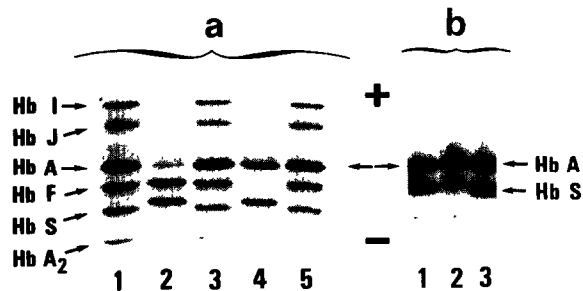


Fig.1. (a) IEF of the propositus and the mother's hemolysates. Lane 2, propositus, Hb D Roseau-Pointe à Pitre/ $\beta^+$  thalassemia (age 2 months). Lane 4, mother, Hb A/Hb D Roseau-Pointe à Pitre. Lanes 1, 3, 5, controls. (b) Cellulose acetate strip electrophoresis. Lane 1, Hb A/Hb D Korle-Bu; lane 2, Hb A/Hb D Roseau-Pointe à Pitre; lane 3, Hb A/Hb S.

Table 1

Amino-acid composition of Hb Roseau-Pointe à Pitre  $\beta$ T10 compared to that of normal  $\beta$ T10

Residue	$\beta$ T10 Hb R.PàP. measured amino-acid molar ratio	$\beta$ T10 Hb A expected amino-acid molar ratio
Gly	1.90	1
Thr	1.90	2
Phe	1.00	1
Ala	1.20	1
Leu	1.80	2
Ser	1.10	1
Glu	0.00	1
His	0.90	1
Cys	0.90	1
Asp	1.20	1
Lys	1.00	1

mother's whole blood  $P_{50}$  was 38 mmHg (normal range, 27–29), with a 2,3-DPG level of 17.3  $\mu$ M/g Hb (normal range, 13–17). The corresponding values were 35 mmHg and 17.7  $\mu$ M/g Hb, respectively, in the propositus.

### 3.2. Structural studies

Separation of the TPCK-tryptic digest of the abnormal aminoethylated  $\beta$  chain by reverse phase HPLC demonstrated that the abnormality was located in peptide  $\beta$ T10 (fig.2). This peptide was not found at its normal position and appeared more hydrophobic, as it eluted before peptide  $\beta$ T12a. Amino-acid analysis (table 1) of this new peptide  $T_x$  indicated an amino-acid composition corresponding to a peptide  $\beta$ T10 with the exception that the single Glu residue F6 was missing, whereas one additional Gly was present. An identical result was obtained upon amino-acid analysis of the abnormal peptide  $\beta$ T10a. From these data the structure of hemoglobin Roseau-Pointe à Pitre was assigned as  $\beta$ 90 (F6) Glu  $\rightarrow$  Gly. In the published sequence of  $\beta$  gene [12] the codon for Glu  $\beta$ 90 is GAG; hence the probable genetic event is a substitution GAG  $\rightarrow$  GGG (glycine).

## 4. DISCUSSION

The biological features of the propositus are not different from those expected in a  $\beta^+$  thalassemia

trait: very low expression of the  $\beta^A$  compared to the  $\beta^D$  gene at birth and slow disappearance of Hb F after birth. In this case, however, the instability of Hb Roseau-Pointe à Pitre probably also contributes to the relatively high Hb F level observed at 6 months. Hb Roseau-Pointe à Pitre is another illustration of the efficiency of both IEF and standard electrophoretic mobilities for the identification of undescribed variants. IEF showed that the studied Hb D had a  $pI$  very close to 3 rare Hbs D (2): Hb D Korle-Bu, Hb D Punjab, and Hb D Iran. Standard electrophoretic mobilities clearly indicated a distinct, and possibly new pattern according to published data [13]. The most interesting feature was the slower mobility of Hb Roseau-Pointe à Pitre in citrate agar electrophoresis which is the probable result of the disappearance of a negative charge in the vicinity of the central cavity [14]. Instability and low oxygen affinity were further arguments for the identification of the abnormal hemoglobin as a new variant.

According to the three-dimensional hemoglobin model [15] position  $\beta$ 90 (F6) occupies an external position on a helical segment close to the  $\alpha_1\beta_2$  interface. As shown by Ackers and co-workers [16] and earlier investigators, most of the free energy difference between the T and R states is localised in this area, which has been one of the most conserved regions during evolutionary history. In mammals,  $\beta$ 90 Glu is an invariant residue. Hemoglobin Roseau-Pointe à Pitre is the second variant with a substitution located at position 6 of the F helix, the other being Hb Agenogi  $\beta$ 90 Glu  $\rightarrow$  Lys, a hemoglobin with decreased oxygen affinity [17]. The interpretation of the low oxygen affinity in Hb Agenogi postulated a new intrachain salt bridge between the Lys (F6) and the C-terminus that pushes the HC<sub>2</sub>  $\beta$  tyrosine towards its deoxy position [17]. The same explanation cannot apply to Hb Roseau-Pointe à Pitre. Nevertheless the new F6 glycine, by disturbing the structure of the F helix, could affect in a similar way the contacts between the F helix and the C-terminus, favoring the deoxy structure, and introducing instability.

## ACKNOWLEDGEMENTS

This work was supported by grants from Institut National de la Santé et de la Recherche Médicale,

from the Centre National de la Recherche and from the Comité Santé et Développement du Ministère de la Recherche et de la Technologie (MRT). J.D.-G. was supported by a fellowship of the MRT. The authors are grateful to M. Francois for logistical help and to the generous technical assistance of H. Lami, J. Riou and Mrs Paterne.

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