Hemoglobin Roseau-Pointe a Pitre $\alpha_2\beta_2$90 (F6) Glu → Gly:

a new hemoglobin variant with slight instability and low oxygen affinity

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A Dominican neonate carrying a new abnormal hemoglobin, hemoglobin Roseau Pointe-à-Pitre $\alpha_2\beta_2$90 (F6) Glu → 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 β^+ thalassemia trait.

Hemoglobinopathy *β variant-Guadeloupe* *β 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 β gene. Most of these cases are Hb D Korle-Bu or Hb D Punjab traits. Electrophoretical 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 β90 (F6) residue. This variant, homologous to Hb Agenogi Glu⟶Lys β90 (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% β-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_2 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

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

Structural analysis was conducted on the aminoethylated abnormal β chain prepared by CM-cellulose (CM52, Whatman) chromatography [9] and desalted through a Biogel P_2 column (BioRad). Tryptic peptide separation of the Hb Roseau-Pointe à Pitre β 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_0 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_0, 15.1%; Hb F, 17.3%; Hb A_2, 5.3%. The mother carried the trait (fig.1a) (Hb D, 36%; Hb A_2, 2.2%) and the father had the typical biological features of a β thalassemia trait (Hb A_2, 5.6%; MCV, 78 Fl). It was concluded that the propositus carried an association of Hb D and β^+ thalassemia traits. Hematological data for the propositus were at the same period: RBC, 4.54 × 10^{12}/l; Hb, 10.8 g/dl; PCV, 31.6%; MCV, 69.6 fl; MCH, 23.2 pg; reticulocytes, 33 × 10^9/l. The mother had a moderate microcytic anemia (RBC, 4.77 × 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 α 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.

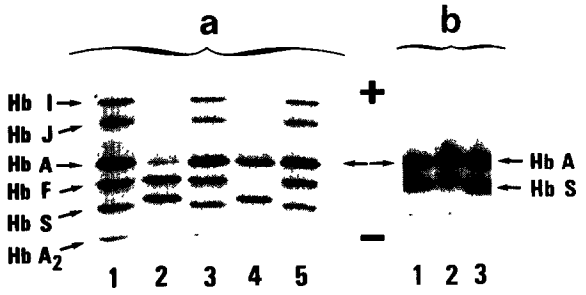

Fig.1. (a) IEF of the propositus and the mother's hemolysates. Lane 2, propositus, Hb D Roseau-Pointe à Pitre/β^+ thalassemia (age 2 months). Lane 4, mother, Hb A/Hb D Roseau-Pointe à Pitre. Lanes 1, 3, 5, controls. (b) Cellulose acetate strip electrophoresis. Lane 1, Hb A/Hb D Korle-Bu; lane 2, Hb A/Hb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

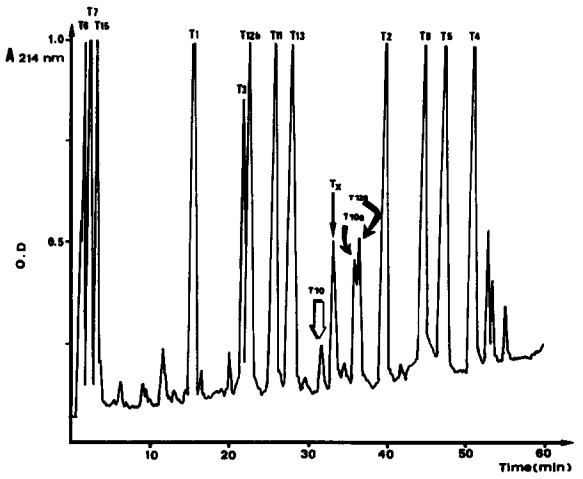

Fig.2. Elution profile of the tryptic digest of the aminoethylated β chain of hemoglobin Roseau-Pointe à Pitre by reverse phase HPLC. The amino acid composition of peptide T_x is given in table 1.

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

Table 1

Amino-acid composition of Hb Roseau-Pointe à Pitre βT10 compared to that of normal βT10

Residue	βT10 Hb R.PàP. measured amino-acid molar ratio	βT10 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 μM/g Hb (normal range, 13–17). The corresponding values were 35 mmHg and 17.7 μM/g Hb, respectively, in the propositus.

3.2. *Structural studies*

Separation of the TPCK-tryptic digest of the abnormal aminoethylated β chain by reverse phase HPLC demonstrated that the abnormality was located in peptide βT10 (fig.2). This peptide was not found at its normal position and appeared more hydrophobic, as it eluted before peptide βT12a. Amino-acid analysis (table 1) of this new peptide T_x indicated an amino-acid composition corresponding to a peptide βT10 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 βT10a. From these data the structure of hemoglobin Roseau-Pointe à Pitre was assigned as β90 (F6) Glu⟶Gly. In the published sequence of β gene [12] the codon for Glu β90 is GAG; hence the probable genetic event is a substitution GAG⟶GGG (glycine).

4. DISCUSSION

The biological features of the propositus are not different from those expected in a β^+ thalassemia trait: very low expression of the β^A compared to the β^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 electrophoretical mobilitites for the identification of undescribed variants. IEF showed that the studied Hb D had a p*I* very close to 3 rare Hbs D (2): Hb D Korle-Bu, Hb D Punjab, and Hb D Iran. Standard electrophoretic mobilities clearly indicted 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 β90 (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, β90 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 β90 Glu⟶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_2 β 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.

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REFERENCES

[1] Miyati, T., Suzuki, H., Ohba, Y. and Shibata, S. (1966) Clin. Chim. Acta 14, 624–631.
[2] Basset, P., Beuzard, Y., Garel, M.C. and Rosa, J. (1978) Blood 51, 971–982.
[3] Schneider, R.G. (1978) Crit. Rev. Clin. Lab. Sci. 243, 271.
[4] Alter, B.P., Goff, S.C., Efremov, D.G., Gravely, M.E. and Huisman, T.H.J. (1980) Br. J. Haematol. 44, 527–534.
[5] Betke, K., Marti, H.R. and Schlicht, I. (1959) Nature 184, 1877–1888.
[6] Huisman, T.H.J., Schroeder, W.A., Brodie, A.N., Maison, S.M. and Jakway, J. (1974) J. Lab. Clin. Med. 83, 657–702.
[7] Carrell, R.W. and Kay, R. (1972) Br. J. Haematol. 23, 615–619.
[8] Rose, Z.B. and Liebowitz, J. (1970) J. Biol. Chem. 245, 3232–3241.
[9] Clegg, J.B., Naughton, M.A. and Wheatherall, D.J. (1966) J. Mol. Biol. 19, 91–108.
[10] Wilson, J.B., Lam, H., Pravatmuang, P. and Huisman, T.H.J. (1979) J. Chromatogr. 179, 271–290.
[11] Schneider, R.G. and Barwick, R.C. (1978) Hemoglobin 2, 417–435.
[12] Lawn, R.M., Efstratiadis, A., O'Connell, C. and Maniatis, T. (1980) Cell 21, 647–651.
[13] Barwick, R.C. and Schneider, R.T.G. (1981) Tex. Rep. Biol. Med. 40, 143–156.
[14] Bunn, F.H. and McDonald, M.J. (1983) Nature 306, 498.
[15] Bolton, W. and Perutz, M.F. (1970) Nature 228, 551.
[16] Pettigrew, D.W., Romeo, P.H., Tsapis, A., Thillet, J., Smith, M.L., Turner, B.W. and Ackers, G.K. (1982) Proc. Natl. Acad. Sci. USA 79, 1849–1853.
[17] Imai, K., Morimoto, H., Kotani, M., Shibata, S., Miyaji, T. and Matsumoto, K. (1970) Biochim. Biophys. Acta 200, 197–202.