POLYMORPHISM OF HUMAN FETAL HAEMOGLOBIN STUDIED BY ISOELECTRIC FOCUSING

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1. Introduction

Human fetal haemoglobin (Hb F, $\alpha_2\gamma_2$) is a mixture of different molecular species. Two of them differ in position 136 of the γ chain which is occupied either by glycine ($^G\gamma$ chain) or by alanine ($^A\gamma$ chain) [1]. These chains are the products of two closely linked genes present in all normal individuals [1,2].

A variant of Hb F, Hb F Sardinia ($\alpha_2{}^A\gamma_2$ 75 Ile → Thr) [3] is of unusual high frequency in various populations at birth [4,5]. Very recent results of peptide analysis suggested that the γ75 Thr chain is a variant produced by the $^A\gamma$ locus [6,7] and corresponds to a genetic polymorphism.

The study of this polymorphism is made difficult by the complexity of the methodology involved in peptide analysis [1,4,5]. In contrast, we report that isoelectric focusing (IEF) on acrylamide slabs using a shallow gradient of laboratory made ampholytes (pH 6.5–7.8) allows to separate Hb F γ75 Thr from Hb F γ75 Ile. It is a reliable and a simple method for the study of such polymorphism. We provide a direct evidence that the γ75 Thr chain variant is produced by the $^A\gamma$ locus.

2. Materials and methods

The isoelectric focusing on thin layer has been described [8]. The main modification is the use of ampholytes synthesized and fractionated according to [9,10]. The gel (125 × 115 × 0.5 mm) was prepared on pre-treated Mylar (Marine Colloids, Rockland, ME).

The selected gradient was pH 6.5–7.8. The migration was performed at 10°C on a Pharmacia apparatus using a constant power of 16 W (1.0 W/ml gel). When focusing had reached completion in 5 h, the gel was placed in 20% trichloroacetic acid for 5 min, rinsed with water and scanned at 415 nm with the Supercellomatic (Sebia, Issy les Moulineaux). Finally, the gel was transferred onto filter paper (Whatmann 3MM) and dried overnight at room temperature for storage.

To determine the nature of the residue present in position γ75, the Hb F fractions were eluted from the gel. The globins were submitted to tryptic digestion and to fingerprinting on paper [4] and on thin layer [11].

The identification of the residues present in position γ136 of the two Hb F fractions was determined by three different techniques: fingerprint of the γ chains cleaved by cyanogen bromide [12]; isoelectric focusing of globin chains in urea and Nonidet P40 [13]; cellulose acetate electrophoresis of globin chains in urea and Nonidet P40 [14].

3. Results

Fig.1 shows the isoelectric focusing pattern of blood samples of two patients homozygous for β thalassemia. Sample 1 exhibited only one main Hb F fraction while sample 2 contained two main fractions called anodic (F^+) and cathodic (F^-). The scanning of the gel at 415 nm showed that the Hb

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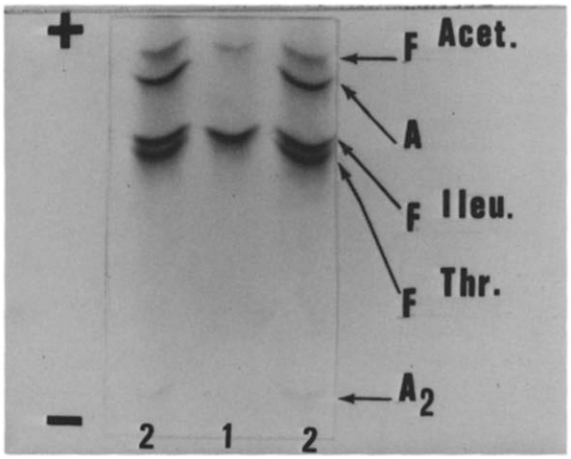

Fig.1. Isoelectric focusing pattern of the haemoglobins of two patients homozygous for β thalassemia.

F^- fraction represented 45% of the total of the two Hb F fractions.

The fingerprints on thin layer shown in fig.2 were identical to those performed on paper and they provided evidence that the anodic fraction Hb F^+ exhibited only the peptide γT-9 Ile while the cathodic fraction Hb F^- contained mostly the γT-9 Thr peptide, indicating that the Hb F^+ fraction contained the γ75 Ile chain and the Hb F^- fraction contained the γ75 Thr chain. These results were confirmed by amino acid analysis of the γT-9 peptides eluted from the fingerprint.

All three techniques used to determine the nature of the residue present in position γ136 showed that in sample 2 the cathodic (Hb F^-) fraction contained only the $^{A}\gamma$ chain and the Hb F^+ contained only the $^{G}\gamma$ chain. In contrast, patient 1 exhibiting only Hb F^+ had both $^{A}\gamma$ and $^{G}\gamma$ chains.

All these results concerning the γ chain heterogeneity indicated that patient 2 exhibited two types of γ chains: γ136 Gly 75 Ile ($^{G}\gamma$ 75 Ile) in the Hb F^+ fraction; and γ 136 Ala 75 Thr ($^{A}\gamma$ 75 Thr) in the Hb F^- fraction. Patient 1 exhibited two γ chains differing only in position 136, γ 136 Gly 75 Ile ($^{G}\gamma$ 75 Ile) and 136 Ala 75 Ile ($^{A}\gamma$ 75 Ile), both present in Hb F^+. Consequently patient 2 can be considered to be homozygous for the ($^{A}\gamma$ 75 Thr and patient 1 was homozygous for the ($^{A}\gamma$ 75 Ile). Concordant results obtained for 5 selected patients homozygous for β thalassemia are summarized in table 1 and provide direct evidence that IEF is able to separate the fetal haemoglobins differing only in position γ 75 and that the γ 75 Thr gene is allelic at the $^{A}\gamma$ locus.

In addition acetylated Hb F, the minor fraction of Hb F shown in fig.1, contained all different types of

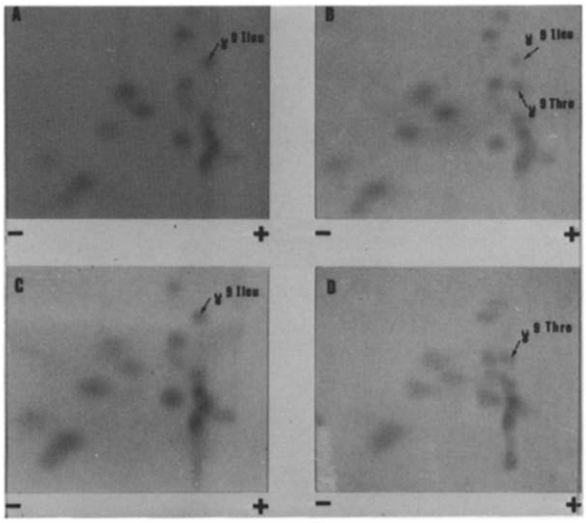

Fig.2. Fingerprint on thin layer of globin F. (A) Total globin F of patient 1. (B) Total globin F of patient 2. (C) The anodic fraction F^+ of patient 2. (D) The cathodic fraction F^- of patient 2. γ9 represent the tryptic peptide γT-9 the γ chain which contains the residue γ75.

Table 1
Types of Hb F and of γ chains of 5 patients homozygous for β thalassemia

	IEF of haemoglobin		Tryptic peptide	IEF of globin chains	
	Fγ75 Thr (%)	Fγ75 Ile (%)	γT-9 Thr (%)	A_γ (%)	G_γ (%)
1	0	100	0	44	56
2	45	55	45	46	54
3	0	100	0	50	50
4	55	45	50	51	49
5	31	69	33	30	70

γ chains present in the main Hb F fractions indicating that IEF allowed one to distinguish the main fractions of Hb Hb Fγ 75 Thr and Hb F γ 75 Ile but not their acetylated derivatives.

In conclusion, IEF made it possible to separate the Hbs F differing by an electrophoretically silent substitution Hb F γ 75 Thr and Hb F γ 75 Ile. Analytical IEF could permit rapid progress in the understanding of $^{A}\gamma$ gene polymorphism in various populations and the expression of the $^{A}\gamma$ 75 Thr gene during human development or in various conditions which reactivate the γ genes after birth. In addition, in the heterozygous state, the Hb Fγ 75 Thr considered as a variant, allows one to study the linkage between the $^{A}\gamma$ gene and β gene defects such as Hb S or β thalassemia and also the linkage of the $^{A}\gamma$ gene to the genetic defects of the heterocellular persistence of fetal haemoglobin which have been found to be linked to mutations of the β gene [15,16].

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