A new silent hemoglobin variant in a black family from French West Indies

Hemoglobin Le Lamentin α20 His → Gln

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A new abnormal hemoglobin Hb Le Lamentin α20 (B1) His→Gln was discovered during a survey of cord blood from the French West Indies (Martinique). This variant displays an electrophoretic pattern similar to that of Hb A but can be isolated by isoelectric focusing (IEF) and Biorex 70 chromatography. Family studies showed the presence of this hemoglobin variant in the father and in two of his three children. Hematological data from the carriers were normal.

Hemoglobin Le Lamentin α20 (BI) His→Gln *Silent Hb variant* *(Corol blood)* *(Isoelectric focusing)*

1. INTRODUCTION

We described in the present paper a new electrophoretically and clinically silent hemoglobin variant discovered during a mass screening of cord blood by isoelectric focusing. The great majority of clinically silent variants have been detected by conventional procedures as a result of routine screening of selected populations and because they presented a clear difference in their electrophoretic pattern.

In the present case isoelectric focusing was the only electrophoretic method giving a clear separation of the variant and therefore permitting its detection.

2. MATERIALS AND METHODS

Hematological studies were done by routine procedure using a Coulter counter model S. Electrophoretic studies were performed on cellulose acetate strip (Sebia) with Tris, EDTA, borate buffer (pH 8.6). Isoelectric focusing was done on thin-layer polyacrylamide gel as previously described [1]. Citrate agar electrophoresis of Hb at pH 6.0, globin chain electrophoresis in urea 8 M at pH 6.0 and pH 9.0 were performed according to standard procedures.

Whole blood affinity for O_2 was determined on a Hem-O-Scan (Aminco, Silver springs MD). Erythrocyte 2,3-DPG-concentration was measured according to the method in [2]. Stability of Hb was tested by isopropanol test [3]. Hemoglobin A_2 was evaluated by microchromatography on DE-52 column with glycine (0.2 M) KCN buffer [4] and Hb F by alkali denaturation as described in [5].

The abnormal fraction was isolated by chromatography on Biorex 70 (Biorad) [6] according to a modification of the method in [7].

After deheminization by acid acetone precipitation, the chains were separated as in [8] and made free of urea on a Biogel P_2 (Biorad) column. The tryptic peptides of the α-amino ethylated chain were separated by finger-print on silicagel thin layer plates [9], eluted, submitted to hydrochloric acid hydrolysis. The amino acid composition was determined on a Biotronik 6000 1E (Biotronik, Munich).

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3. RESULTS

The abnormal Hb was discovered, during a routine screening performed by IEF [10] for Hb S and C on the new born negroes at Fort de France, Martinique. Family study showed the presence of this variant in the father and in an another child. No clinical or hematological abnormalities were found in association with this hemoglobin. The isopropanol test was negative. The P50 of whole blood of the father was 31 mmHg (n = 28 ± 1) and the 2,3-DPG level was normal: 12.85 μmol/g Hb (n = 15 ± 2). Identification and structural studies were performed on a father's sample. At alkaline pH, the electrophoretic pattern was indistinguishable from that of the normal one. The variant migrated as Hb A on citrate agar electrophoresis (pH 6.00). By IEF the abnormal component, representing 20% of the total, focused slightly more anodically than Hb A_{Ic}.

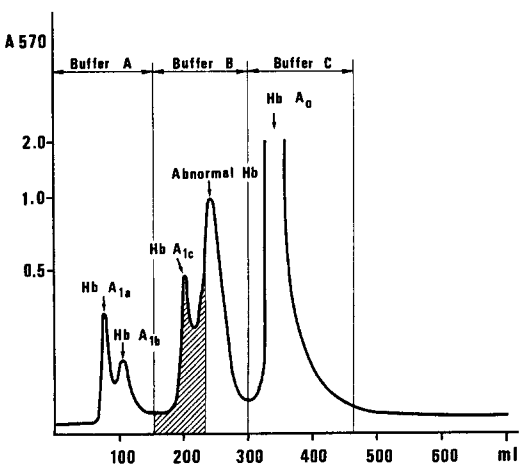

Fig.1. Purification of the abnormal fraction by column chromatography 800 mg of hemolysate were separated on a column of Biorex 70 (200–400 Msh) diam. 2.5 × 18 cm, equilibrated with a sodium phosphate buffer (0.048 M) pH 6.75. The fractions which contain the 'non-heme protein' (N.H.P.) Hb A_{Ia} and Hb A_{Ib} were eluted with the equilibrium buffer A. The other fractions were eluted stepwise using NaCl concentration: 0.1 N NaCl for Hb A_{Ic} and Hb Le Lamentin (Buffer B); 0.3 N NaCl for Hb A_0 and Hb A_2 (Buffer C). Control by IEF of the hatched fraction showed a single band of pure Hb Le Lamentin.

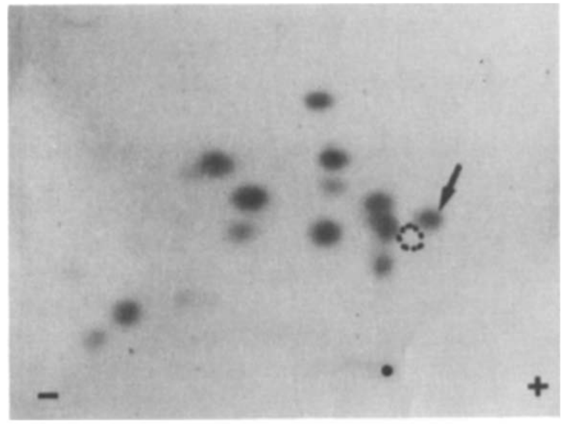

Fig.2. Finger print of the tryptic digest of αAE Le Lamentin at pH 6.4 on a silica gel layer plate. The dotted circle shows that the spot of the α^{A}T4 peptide is absent. The arrow indicates the spot of the new peptide αT4.

Globin chain electrophoresis in urea 8 M at pH 9.00 demonstrated the presence of an abnormal α chain which was not detected at pH 6.0. The abnormal hemoglobin could be obtained pure by chromatography on Biorex 70 (fig.1). The finger print map of the α variant tryptic peptides is shown on fig.2. The normal peptide αT4 is absent and a more anodic new peptide is visible. Specific staining of this new peptide gave a positive reaction for tyrosine and arginine and a negative one for histidine.

The amino acid composition of this peptide (table 1) showed an αT4 stoichiometry except for

Table 1

Amino acid composition of peptide αT4

	Hb Le Lamentin	Hb A
Glu	4	3
Gly	3	3
Ala	4	4
Val	1	1
Leu	1	1
Tyr	1	1
His	0	1
Arg	1	1

the replacement of the His 20 by a glutamine or a glutamic acid.

Evidence that hemoglobin Le Lamentin αT4 peptide contains glutamine rather than glutamic acid resulted from the following considerations: Glu instead of Gln in the αT4 peptide would produce a more anodic position in urea electrophoresis of the chains at pH 6.0; the presently accepted coding assignment allows His→Gln in one step transition but exclude His→Glu.

The substitution His→Gln is moreover the only compatible one, according to genetic code data with only one base replacement. Since the codon for His α20 is CAC [11], the substitution is likely CAC→CAA.

4. DISCUSSION

We have described a new variant of the α chain α20 His→Gln which is clinically and biologically silent. The standard electrophoretic procedure failed to detect the abnormal band.

This result gives another example of the high resolving power of IEF screening method [1]. The His→Gln transition has not been previously described in α variants despite the presence of several cases involving a histidine substitution. In contrast, it has been observed in two instances in the β-chain: Hb St Etienne β92 His→Gln [12] and Hb Malmo β97 His→Gln [13]. The abnormal peptides of the three variants exhibited approximately the same difference of mobility compared to their normal control. But in IEF, Hb Le Lamentin migrates approximately as Hb Malmo in contrast to Hb St Etienne which is not separated from Hb A except for its deheminized form. This is likely a consequence of the rather external position of α20 and β97.

Another substitution of His α20 has been described in Hb Necker-Enfants Malades [14]. Several similarities are present between Hb Le Lamentin and Hb Necker-Enfants Malades. They have been found in two subjects originated from French West Indies, they do not produce clinical abnormality and are well purified by chromatography on Biorex 70.

According to the results obtained by Wischner [15] the residue His α20 may participate in an ionic bond with Glu β22 of a neighboring molecule during the polymerisation of the Hb's tetramers. Further work is underway to test this hypothesis.

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