Hb HENRI MONDOR: β26 (B8) GLU → VAL: A VARIANT WITH A SUBSTITUTION LOCALIZED AT THE SAME POSITION AS THAT OF HbE β26 GLU → LYS

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Received 8 November 1976

1. Introduction

The present report describes a new hemoglobin which has been observed in a young African girl. This new variant which has been identified as β26 (B8) Glu → Val is a homologue of HbE β26 (B8) Glu → Lys [1], the third most common variant of hemoglobin. A slight instability of hemoglobin and a hypochromic anemia were also detected in the subject.

2. Materials and methods

Routine hematological data were obtained using standard techniques. The isopropanol solubility test was performed as previously described [2]. Hemolysates were prepared according to Drabkin [3]. Hemoglobin electrophoresis was carried out on cellulose-acetate strips using Tris–EDTA–borate buffer, at pH 8.6 [4] and on agar-gel using citrate buffer, at pH 6.2 [5]. Chromatography of the whole hemolysate was performed on a column of DEAE–Sephadex as described by Huisman and Dozy [6]. Electrophoresis of the α- and β-chains was performed as described earlier [7]. Globin was prepared by acid–acetone precipitation at −20°C and then separated into α- and β-chains by carboxymethyl-cellulose (CM 52) chromatography in 8 M urea buffers [8]. Tryptic peptides from amino-ethylated chains were isolated either by fingerprinting on thin-layer silica-gel plates [9] or by chromatography on a column of Aminex A 5 [10]. Amino acid analysis was carried out on a Jeol JLC 5 A H amino acid analyser. Sequential analysis of amino acid residues was done by the manual Edman degradation following the procedure described by Weiner et al. [11] with slight modifications.

3. Results

3.1. *Case report*

The new hemoglobin variant was discovered in a 9 year old African girl. Her blood picture and Hb concentration revealed a hypochromic microcytic anemia (table 1).

3.2. *Structural studies*

Electrophoresis of freshly prepared hemolysates on cellulose acetate strips in Tris–EDTA–borate buffer, at pH 8.6, demonstrated a band of hemoglobin migrating more slowly than the HbA fraction

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Table 1
Summary of hematological data from the patient with Hb Henri Mondor

Hb (g/100 ml)	Erythrocytes ($\times 10^6$/mm^3)	Ht (%)	Reticulocytes ($\times 10^3$/mm^3)	Serum Fe (μg/100 ml)
7.5	3.7	24	20	47

and representing 37.5% of the total hemoglobin (fig.1).

The HbA_2 level was normal (2.5%). A slight instability was demonstrated by the isopropanol solubility test. The abnormal hemoglobin migrated as HbA on citrate-agar electrophoresis, at pH 6.2. Electrophoresis of the total hemolysate in 6 M urea buffer, at pH 6.00, revealed the presence of abnormal β-chains. Pure Hb Henri Mondor was obtained by DEAE–Sephadex column chromatography and was eluted at pH 8.05.

Analytical fingerprints of tryptic digests of the AE β Henri Mondor chain showed the absence of the normal βT3 peptide and revealed the presence of a new peptide with a greater R_f localized near the βT5 peptide (fig.2).

The abnormal peptide was eluted from preparative fingerprints with 6 N HCl and hydrolysed for 22 h at 110°C. Its amino acid composition (table 2) showed that the abnormal peptide differed from normal βT3 in having only one residue of glutamic acid instead of two, together with an additional valine residue.

Table 2
Amino acid composition of the βT3 peptide from Hb Henri Mondor

Amino acid	Molar ratios	
	Found	Normal βT3
Arginine	0.9	1
Aspartic acid	2.1	2
Glutamic acid	1.0	2
Glycine	3.1	3
Alanine	1.1	1
Valine	3.9	3
Leucine	1.0	1

In order to confirm which one of the two residues of glutamic acid was replaced, large amounts of the abnormal peptide were prepared by column chromatography on Aminex A_5. Further purification was

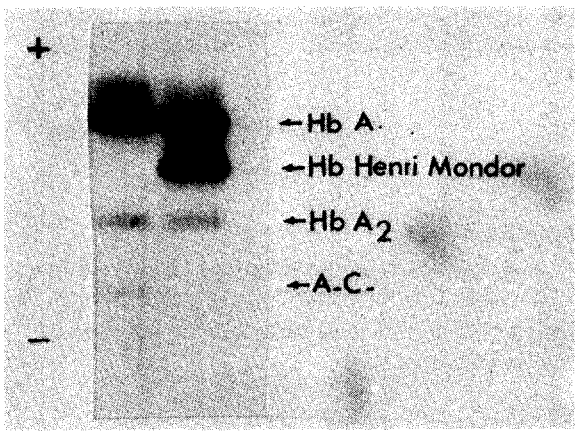

Fig.1. Electrophoresis on cellulose-acetate strips (Tris–EDTA–borate buffer, pH 8.6) of hemolysates prepared from the following (left to right): (a) normal adult (b) blood from the proband.

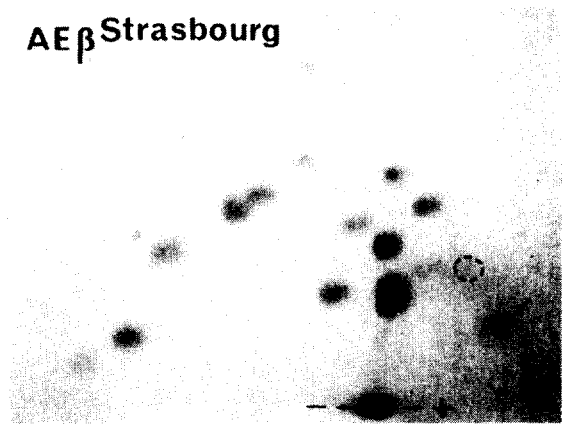

Fig.2. Fingerprint of tryptic peptides from the AE β-chain of Hb Henri Mondor. The arrow shows the position of the new βT3 peptide; the dotted circle indicates the position occupied by βT3 peptide in the control.

done on a column of AGI X 2. Edman degradation of the abnormal peptide indicated that the glutamic acid residue in position 26 of the β-chain was replaced by one of valine. The actual sequence of the βT3 of Hb Henri Mondor is:

$$\overset{18}{\text{Val}} - \overset{19}{\text{Asn}} - \overset{20}{\text{Val}} - \overset{21}{\text{Asp}} - \overset{22}{\text{Glu}} - \overset{23}{\text{Val}} - \overset{24}{\text{Gly}} - \overset{25}{\text{Gly}} - \overset{26}{\text{Val}} - \text{Ala} - \text{Leu} - \text{Gly} - \text{Arg}$$

4. Discussion

The amino acid substitution of Hb Henri Modor β26 (B8) Glu → Val which we have described is located at the B8 residue; this residue does not vary in any of the normal polypeptide chains of human hemoglobin (α, β, γ, δ). The substitution present in Hb Henri Mondor occurs in the same position as that of HbE β26 (B8) Glu → Lys [1], the third most common variant of hemoglobin. Several reviews have listed HbE among the hemoglobin variants exhibiting abnormally low oxygen affinity [12] but the studies performed by Bunn et al. [13] did not confirm these earlier reports. Consequently more detailed examination of the oxygen equilibrium of Hb Henri Mondor is needed in order to compare it with that for HbE. It is interesting to note that a slight anemia and a slight instability of the molecule was observed in the patient with Hb Henri Mondor.

Hemoglobin Fort Worth α27 (B8) Glu → Gly [14] and Hb Spanish Town α27 (B8) Glu → Val [15] are the homologues of Hb Henri Mondor on the α chain. The hemolysate containing Hb Fort Worth showed a slightly increased hemoglobin precipitation as did the hemolysate containing Hb Henri Mondor and the hematologic data indicated the same hypochromia and microcytosis. Hb Spanish Town did not however exhibit any instability.

Acknowledgements

This work was supported in part by l'Institut National de la Santé et de la Recherche Médicale, grant No. 73 502 72, by la Délégation Générale à la Recherche Scientifique et Technique, grant No. 72 702 82, by la Fondation pour la Recherche Médicale Française and by l'Université de Paris Val de Marne. P.E.A. Machado is boursier de la FAPESP 75 0944. The authors wish to acknowledge Dr J. Chapman for careful assistance in reviewing the manuscript.

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