HEMOGLOBIN J LOME β 59 (E3) Lys → Asn

A new fast moving variant found in a Togolese

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1. Introduction

In a systematic survey of 2700 persons for abnormal haemoglobin at the Medical School of Lome (Republic of Togo), 16% HbS and 7% HbC were found and the rare mutants HbK Woolwich and Hb Korle Bu were also observed. In addition a new fast moving variant named HbJ Lome, β59 (E3) Lys → Asn, was identified. The presence of this new haemoglobin in an 11 year old girl was without clinical or haematological consequences (rbc 5.4 × 10^{12}/litre, haemoglobin 16.7 g/dl, haematocrit 0.49 and MCV 94 fl). The abnormality was also found in the mother and in some other members of the family.

2. Materials and methods

Heparinised blood was lysed by a routine procedure. Electrophoresis was at pH 8.6 in Tris–EDTA–borate buffer on cellulose acetate plates. Isoelectric-focusing in polyacrylamide gel was as in [1] modified as in [2].

The abnormal haemoglobin was separated from HbA_1 by DEAE–Sephadex chromatography using a linear gradient from pH 7.8–6.8 of Tris–HCl 0.05 M buffers [3]. Globin was prepared by the acid acetone methods and the chains separated as in [4].

The aminoethylated tryptic peptides were separated on cellulose or silica gel thin-layer plates [5,6]. After ninhydrin staining and elution, peptides were hydrolysed with HCl and the amino acid compositions determined on a Chromaspek Amino-Acid Analyser (Rank Hilger).

The β chain was cleaved by cyanogen bromide and the peptides β_{CB1} and β_{CB2} separated by gel chromatography on a 150 × 2.5 cm column of Sephadex G-50.

Peptide β_{CB2} was sequenced according to [8] using an Anachem APS 2400 solid-phase sequencer, the peptide being attached to AEAP glass as in [9]. The PTH derivatives were identified by miniaturized thin-layer chromatography [10].

The oxygen affinities of intact cells and purified phosphate-free haemoglobins were measured as in [11].

Auto-oxidizability was measured at 42°C in Tris–HCl 0.05 M, NaCl 0.1 M buffer, pH 7.4, by following the decrease of A_{572} and the increase of A_{630}. Methaemoglobin was obtained by addition of a slight excess of potassium ferricyanide.

3. Results and discussion

3.1. *Structural studies*

Electrophoresis at pH 8.6 of the lysate showed the presence of a fast-moving component migrating like

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HbJ and amounting to approx. 50% of the total haemoglobin. Its isoelectric point was 6.67 ± 0.01 and it could not be distinguished from HbJ Baltimore β16 (A 13) Gly → Asp [12].

By CM-cellulose chromatography of the globin, in urea, a fast-eluting β chain was isolated, the fingerprint of the tryptic digest of which showed the absence of the spot corresponding to peptide βT6 (fig.1). The most probable reason for the disappearance of this dipeptide is substitution of either Lys β59 or Lys β61, giving rise to an abnormal and elongated βT5 or βT7.

The position of βT5 was normal on the fingerprint but its composition was abnormal since it contained two extra residues, Val and Asx (table 1). From the sequence of this part of the molecule (table 2), this amino acid composition can be explained by the replacement of Lys β59 by an Asx, tryptic cleavage then occurring after Lys β61. A single base mutation would require lysine to be replaced by Asn.

The sequence of this part of the molecule was determined starting with 5 mg freeze-dried β_{CB2} peptide. It was possible to bind sufficient peptide to the AEAP glass to determine without ambiguity the sequence of the first seven residues. When normal β_{CB2} was sequenced by this method, Asn was found at the second step and there was still some Asn remaining at the third step but none at the fourth. With Hb Lome β_{CB2} a clear spot of Asn PTH was found at the fourth step and some Asn also remained at the next step. This shows that the β59 Lys had been replaced by Asn.

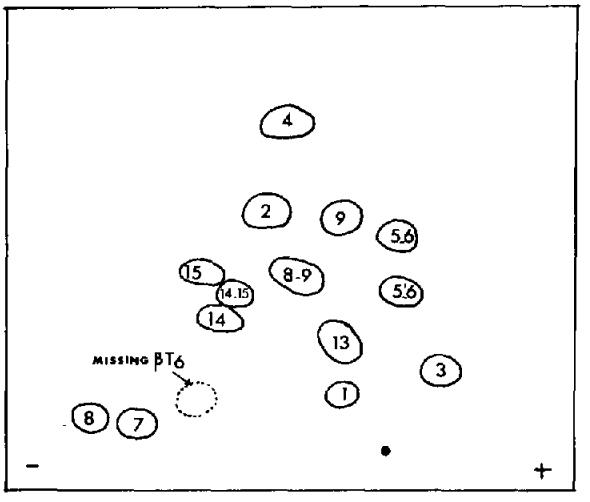

Fig.1. Finger-print of $\beta_{J\ Lome}$ tryptic digest. Peptide βT6 is missing and at the place of normal βT5 a peptide containing two extra residues (β_{T5-6}) was found.

Table 1
Amino acid composition of the abnormal peptide

	Residues found	Normal βT5	Normal βT6
Asp	4.3	3	
Thr	1.1	1	
Ser	2.0	2	
Glu	1.1	1	
Pro	1.9	2	
Gly	1.8	2	
Ala	0.9	1	
Val	2.1	1	1
Met	+	1	
Leu	1.0	1	
Phe	2.4	3	
Lys	0.9	1	1

3.2. *Functional studies*

In intact cells suspended in isotonic phosphate buffer, the oxygen affinity was found normal. The P_{50} and the cooperativity of the phosphate-free abnormal component were identical to those of HbA_1.

Since the structural abnormality is located on helix E, near the distal histidine, the auto-oxidizability was checked. Hb Lome is oxidized much faster than HbA_1. After 5 h incubation at 42°C, in presence of atmospheric oxygen, 60% Hb Lome was oxidized against 42% HbA_1. This result is consistent with the hypothesis that the distal histidine helps to keep the heme-iron in the reduced form [13].

HbJ Lome is the third variant of position 59 described, the two others are HbI High Wycombe [14] and HbJ Kaohsiung [15] in which the lysine is replaced respectively by a glutamic acid and a threonine. The rate of auto-oxidizability was not studied in these abnormal hemoglobins.

Table 2
Sequence of the β T 5-6 peptide of Hb A and Hb J. Lome

	41			45						50
βA	Phe	– Phe	– Glu	– Ser	– Phe	– Gly	– Asp	– Leu	– Ser	– Thr–
βJ Lome		–	–	–	–	–	–	–	–	–
	←---------- βT5 ----------									
	51				55					60
βA	Pro	– Asp	– Ala	– Val	– Met	– Gly	– Asn	– Pro	– Lys	– Val–Lys
βJ Lome	–	–	–	–	–	–	–	–	Asn	
	----------→ ←βT6 →→									
	——————→				←——————					
	βCB1				βCB2					

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