A NEW UNSTABLE HEMOGLOBIN MUTATED IN β 98 (FG 5) Val → Ala: Hb DJELFA

Gérard GACON, Henri WAJCMAN and Dominique LABIE
Institut de Pathologie Moléculaire, Unité 15 INSERM, CHU Cochin, 24, rue du faubourg Saint-Jacques, 75014 Paris, France

and

Alain COSSON
Service d'Hématologie, CHU Necker, 75015, Paris, France

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1. Introduction

In 1968, on the basis of the three-dimensional model, the unstable hemoglobins have been discussed by Perutz and Lehmann [1]. They demonstrated that the instability of the protein was a consequence of structural modifications located on some definite parts of the molecule among which are in the vicinity of the heme group.

We have proposed recently a tentative systematic classification of hemoglobins mutated in the heme pocket according to the three-dimensional position of the abnormality [2]. The loss of heme, which is know known to be a major sign, was found in significant amounts only in substitutions located on helix F and segment FG.

In this paper, a new unstable hemoglobin belonging to this group is described : Hb Djelfa, in which the β 98 (FG 5) valine is replaced by an alanine.

2. Material and methods

Blood was collected on heparin and hemolysate prepared by routine procedures.

Electrophoresis was done on cellulose acetate in Tris EDTA-borate buffer, pH 8.6. The stability was studied by the isopropanol method [3].

The abnormal component was selectively isolated by *p*-mercuribenzoate (PMB) using a ratio of 8 PMB/tetramer Hb according to the technique of Rosemeyer and Huehns [4]. At 4°C, after an 18 h reaction, the abnormal chain precipitated, while the normal ones remained soluble.

The heme was then removed from the hemoglobin by the acid-acetone method. The β chain was purified from a slight amount of co-precipitated α chain by chromatography on CM cellulose according to Clegg et al. [5] and amino-ethylated [6].

After tryptic digestion an analytical peptide map was made on cellulose thin layer as previously described [7]. For further studies, the peptides were fractionated by ion-exchange chromatography on Beckman PA 35 resin according to Jones [8]. Purification of the abnormal peptide was done with the same system, using AG 50 W-X2 resin.

After hydrolysis by HCl the aminoacid composition of the peptide was determined on a Beckman 120 C aminoacid analyzer.

3. Results and discussion

3.1. *Electrophoretic studies and stability test*

The electrophoresis of the lysate, at alkaline pH, revealed, in addition to normal hemoglobin, approx. 5% of a minor component migrating slightly more anodically than Hb A_2. There was no fetal hemoglobin and the level of Hb A_2 was normal. This minor band was not modified by potassium cyanide, but disappeared after addition of an excess of hemin, indicating that it was a partially deheminized component.

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This electrophoretic pattern was very similar to that observed with Hb Köln [9].

In the presence of 17% isopropanol a clear precipitation was observed within 20 min.

3.2. *Structural studies*

Alkylation by PMB led to precipitation of about 15% of the total hemoglobin. It was proved, by chain separation in urea, to be 90% pure β chain, with normal chromatographic behaviour.

The finger-print of the tryptic digest and the elution pattern on ion-exchange chromatography were both normal.

To avoid a long and tedious systematic study of all the peptides, we referred to the results of our tentative classification [2]. The loss of heme was the major feature of one group described, this group (table 1) including Hb Köln, the most common unstable hemoglobin and other variants all characterized by a structural abnormality located on helix F and segment FG. The first peptides purified by ion-exchange chromatography were therefore βT 10 and βT 11, which correspond to this part of the molecule. In the aminoacid analysis of peptide βT 11, instead of one residue of valine, one residue of alanine was found (table 2), the sequence of peptide βT 11 being then:

Leu–His–Ala–Asp–Pro–Glu–Asp–Phe–Arg
96 98 100 104

instead of:

Leu–His–Val–Asp–Pro–Glu–Asp–Phe–Arg
96 104

Table 1
Abnormal hemoglobins characterized by a loss of heme

Abnormal hemoglobin	Substituted Residue			Ref.
Hb Tours	F3	β 87 Thr	→ O	[1]
Hb Santa Ana	F4	β 88 Leu	→ Pro	[12]
Hb Sabine	F7	β 91 Leu	→ Pro	[13]
Hb St Etienne[a]	F8	β 92 His	→ Gln	[14]
Hb Gun Hill[a]	F7-FG2	β 91-95	→ O	[15]
Hb Köln	FG5	β 98 Val	→ Met	[16]
Hb Nottingham	FG5	β 98 Val	→ Gly	[17]

[a]These two variants are natural semi-hemoglobins unable to bind the heme.

Table 2
Amino acid composition of peptide β T II

	Residues found	Composition of normal β T II
Histidine	1.0	1
Arginine	0.9	1
Aspartic acid	2.1	2
Glutamic acid	1.0	1
Proline	1.0	1
Alanine	1.0	0
Valine	0.1	1
Leucine	0.1	1
Phenylalanine	0.9	1

Hb Djelfa is the third variant of a substitution located at position FG 5, the two others being Hb Köln and Hb Nottingham.

Preliminary studies give evidence of functional abnormalities almost identical to those described for Hb Köln [10]: in intact cells the oxygen affinity is increased and the curve is biphasic. This new variant demonstrates once more that the proximal side of the heme plane is fundamental in the heme–globin binding.

From the methodological point of view, it is interesting to notice that an indication of the altered part of the molecule can be given by various approaches. The most common is a charge or polarity difference of a given peptide, but other properties, like the heme loss, can be useful tools.

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