STRUCTURAL STUDIES OF A NEW HEMOGLOBIN: HbJ Lens, β13(A10) Ala → Asp

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1. Introduction

This new variant was found in a 2 year old French child, obese and unable to walk. Many investigations were done and during hematological examination, a very mild microcytic hypochromic anemia was found. A fast-moving hemoglobin was noted on cellulose acetate strip electrophoresis. It migrated as HbH or HbJ. This abnormal hemoglobin was characterized by structural studies as $\alpha_2\beta_2$ 13(A10)Ala → Asp and was named HbJ Lens.

2. Methods

2.1. *Routine investigation*

Blood was collected in heparin and hemolysate prepared by routine procedures. Electrophoresis was done on cellulose-acetate in 0.058 M Tris–0.15 M glycine buffer (pH 9.5). HbA_2 level was estimated by DEAE-cellulose chromatography in [1]. Isopropanol solubility test was carried out according to [2] and the heat stability test as in [3]. Other hematological studies were performed according to standard methods.

2.2. *Structural studies*

Globin was prepared by the acid acetone precipitation method at −20°C and globin chain electrophoresis was performed in veronal 6 M urea buffer (pH 8.0) as in [4]. The pure abnormal β-chain was obtained by chromatography on carboxymethylcellulose column in 8 M urea according to [5]. After gel filtration on a column (150 × 2.5 cm) of Sephadex G-25 (coarse) in 0.2 M aqueous acetic acid, the α- and β-chains were freeze dried. The abnormal β-chain was *S*-aminoethylated according to [6] and then desalted on a column of Sephadex G-25. Analytical paper fingerprints of a tryptic digest of aminoethylated β-chain were prepared at pH 6.4 according to [7]. The abnormal tryptic peptide was eluted from preparative paper fingerprints with 6 N HCl after previous purification by paper electrophoresis at pH 3.5 and subjected to acid hydrolysis during 24 h. Amino acids analyses were carried out on a Biotronik LC 2000 amino acid analyzer. The same abnormal peptides stained by fluorescamine were eluted from other preparative paper fingerprints by 8% (v/v) aqueous acetic acid. The sequential analysis of amino acid residues was performed by Edman's manual degradation modified as in [8]. Phenyl thiohydantoin amino acid derivatives were identified by thin-layer chromatography in the solvent system of [9] and by HPLC on a C18 μ-Bondapak column as in [10].

3. Results and discussion

3.1. *Hematological data for the HbJ Lens*

The hematological investigation gave the following results: Hb, 13.8 g/dl; PCV, 0.42 l/l; RBC, 5.49 × 10^{12}/l; WBC, 9.5 × 10^9/l; MCV, 73 fl; MCHC, 35 g/dl; MCH, 25 pg; serum iron, 69 g/dl; platelets, 230 × 10^9/l.

3.2. *Electrophoretic studies and stability test*

Electrophoresis of the hemolysate at alkaline pH on cellulose acetate revealed the presence of an abnormal component which migrated more anodically than HbA in a proportion of ~53.3%, probably increased by the presence of HbA_3. This component was characterized as HbJ (fig.1). The proportion of HbA_2 was ~3% and HbA 43.7%. No abnormality was found by isopropanol or heat stability tests. Globin chain electrophoresis in 6 M urea, however indicated the presence of an abnormal βJ chain migrating more anodically than normal β-chain.

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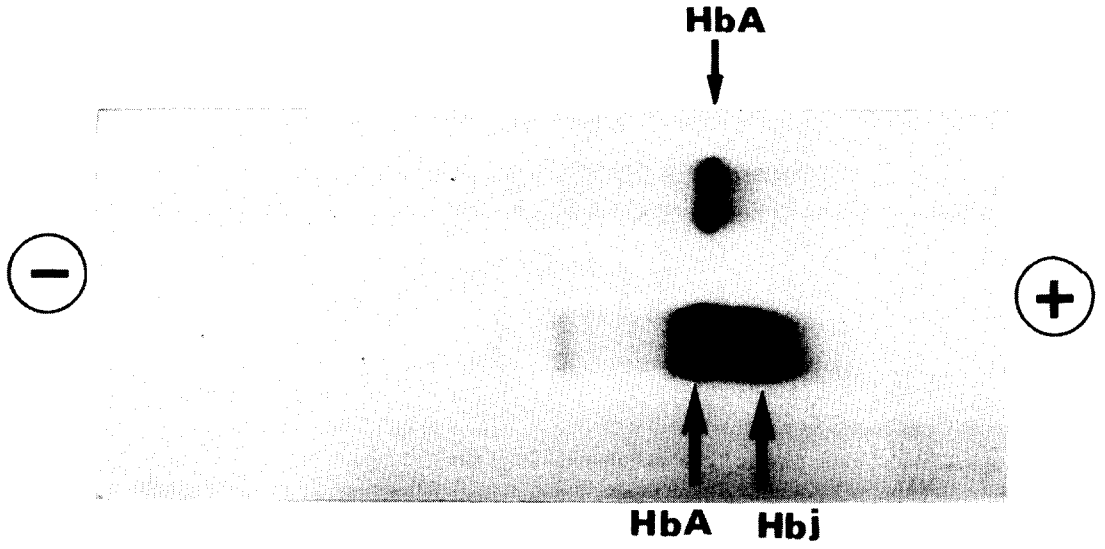

Fig.1. Cellulose acetate electrophoresis of HbJ and HbA hemoglobins in 0.058 M Tris–0.15 M glycine buffer (pH 9.5) as in section 2.

3.3. *Structural studies*

The fingerprints of the abnormal β-chain tryptic peptides showed that the β^{A}TpII (fig.2) which consists of residues 9–17 of the β-chain was absent and stained negatively for tryptophan and very poorly with ninhydrin. We have also found 2 tryptophan-positive peptides: peptide (a) overlapping β^{A}TpIX in the neutral region and peptide (b) between β^{A}TpIII and β^{A}TpXI. Peptide (b) also stained positively for arginine. The amino acid composition of the peptide (a) recovered the whole sequence of the peptide β^{A}TpII except that one alanine was replaced by an extra residue of aspartic acid (table 1). The amino acid composition of peptide (b) suggested that this peptide was an abnormal βTpII–III peptide with addition of one aspartic acid and the absence of an alanine. Since there are two residues of alanine in β^{A}TpII at position β10 and β13, we had to perform the sequence studies and

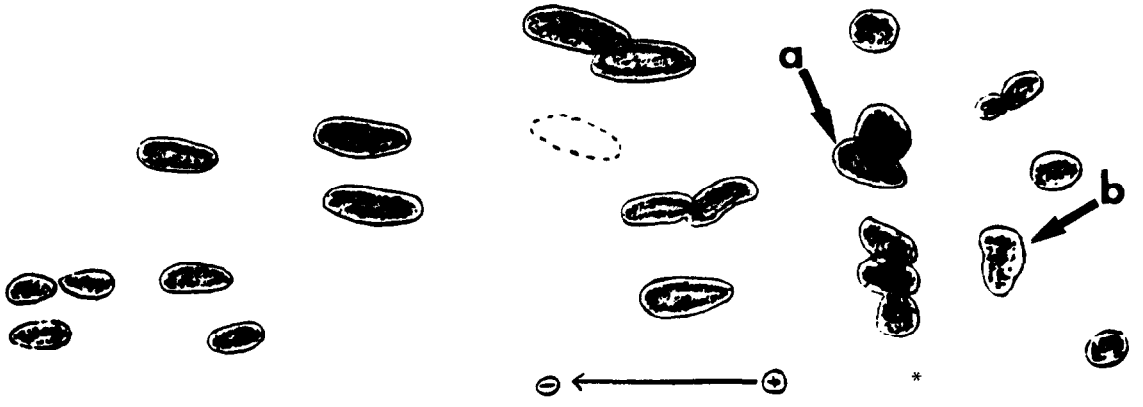

Fig.2. Fingerprints of the tryptic peptides of the amino-ethylated β-chain of HbJ Lens. Electrophoresis at pH 6.4 (60 V/cm, 1 h). Ascending chromatography in the upper phase of pyridine–isoamyl alcohol–water (600:600:700, by vol.) for 20 h. Peptides were located by staining with ninhydrin. (*) Origin (a) position of abnormal βTpII (b) position of abnormal βTpII–III. The broken line indicates the normal position β^{A}TpII of which traces remained on the fingerprint.

Table 1
Amino acid composition of peptide TpII from HbJ Lens

Amino acid	HbJ Lens	Expected for HbA
Asp	0.7	0
Thr	1.0	1
Ser	1.2	1
Gly	1.2	1
Ala	1.2	2
Val	0.8	1
Leu	0.9	1
Lys	0.9	1
Trp[a]	+	+

[a] Tryptophan was identified by UV lamp and by its specific reaction

the following sequence of peptide (a) was found:

Ser–Ala–Val–Thr–Asp–(Leu, Trp, Gly, Lys)
9 10 11 12 13 14 15 16 17

instead of:

Ser–Ala–Val–Thr–Ala–Leu–Trp–Gly–Lys

in the normal peptide. These findings indicated that peptide (a) was the tryptic peptide βTpII in which substitution had occurred at position β13(A10). No hemoglobin with the substitution β13(A10)Ala → Asp has been described before. So we named this abnormal hemoglobin after the city where it was discovered.

4. Discussion

Sequence studies have shown that in β^{J}TpII, alanine at position β13 has been replaced by an aspartic acid residue. This substitution increased the negative charge of the tryptic peptide β^{J}TpII and explained its migration towards the anode. The presence on the fingerprint of an abnormal β^{J}TpII–III peptide indicated that trypsin was not able to split completely the peptide bond between lysine β17 and valine β18. It is not impossible to think that a carboxyl group of aspartic acid at position β13 should be capable of forming a salt bridge with the ε side chain amino group of lysine β17 and therefore make trypsin unable to completely split the protein on the C-terminal side of lysine β17. The substitution in HbJ Lens is at an external site which therefore plays no part in subunit contacts or haem binding. Such an external substitution probably does not affect the properties of hemoglobin tetramer, and the mild microcytic anemia is also probably due to iron deficiency.

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