Hb SHAARE ZEDEK (α56 E5 Lys → Glu)

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1. Introduction

A 2-year-old boy was found to be anaemic. Iron deficiency was diagnosed and on appropriate medication the haemoglobin level rose from 8.5 to 11.5 g/dl. The parents were Persian-speaking Jews who had come to Jerusalem from Kermanshah, Iran, i.e. they belonged to a community in which α-thalassaemia and HbH disease occur. The red cell parameters were suggestive of α-thalassaemia (HbA_2 2.2%, HbF 1%, MCH 24.4 pg and MCHC 30.4 fl). On electrophoresis of the haemoglobin an abnormal band was seen resembling HbH in its position. There were, however, no clinical indications of HbH disease; there was no splenomegaly, no raised reticulocyte count, and no HbH inclusion bodies could be demonstrated in the red cells. The possibility of an abnormal haemoglobin was therefore considered.

2. Methods

The procedures for preparation of haemolysates, separation of haemoglobins by paper and cellulose acetate electrophoresis at pH 8.9, quantitation of haemoglobin fractions, preparation of globin, of tryptic peptides derived therefrom, their two-dimensional separation by high voltage electrophoresis and chromatography, elution of peptides and their analysis have been summarised [1]. Haemoglobins and globin chain separation followed established techniques [2,3].

3. Results

When the haemolysate was submitted to chain separation one band was seen in the position of β^A-chains, one in that of α^A-chains, and a further one in the position of 'fast' α-chains, i.e. α-chains which moved faster towards the negative pole than normal α-chains. On paper and cellulose acetate electrophoresis of the haemolysate the abnormal haemoglobin band migrated in the position of HbH or HbI, i.e. it differed from HbA by the acquisition of 4 negative charges per tetramer or 2 per abnormal α-chain. The abnormal haemoglobin was separated by paper electrophoresis at pH 8.9 and eluted. Globin was prepared and both the abnormal whole globin or, after chain separation (fig.1), the abnormal α-chains were submitted to tryptic digestion. The tryptic peptides were separated by electrophoresis and chromatography to prepare two-dimensional peptide maps (fingerprints). The peptides were visualised by ninhydrin spraying and stained for the presence of specific amino acids (fig.2). The tryptic peptide (Tp)αVI (α41-56) which stains for tyrosine and histidine was absent, and so was αTpVII (α57–60) which would stain for histidine if present. A new peptide was observed which stained both for histidine and tyrosine. The electrophoretic properties of the haemoglobin had indicated that the abnormal chain differed from the HbA chain by acquisition of two negative charges. Providing that only one amino acid residue was substituted this can only arise from the replacement of a positively charged lysine residue by one of a negatively charged glutamic acid. Such a Lys–Glu mutation would also explain the replacement of two tryptic peptides by one. If the C-terminal Lys of αTpVI was replaced by Glu αTpVI and αTpVII would not be separated by tryptic digestion and a larger tryptic peptide αVI–VII would result (fig.3).

Amino acid analysis of the isolated new peptide showed that all the residues expected from αTpVI plus αTpVII were present except that there was found one less Lys and one more Glu (table 1). As the new pep-

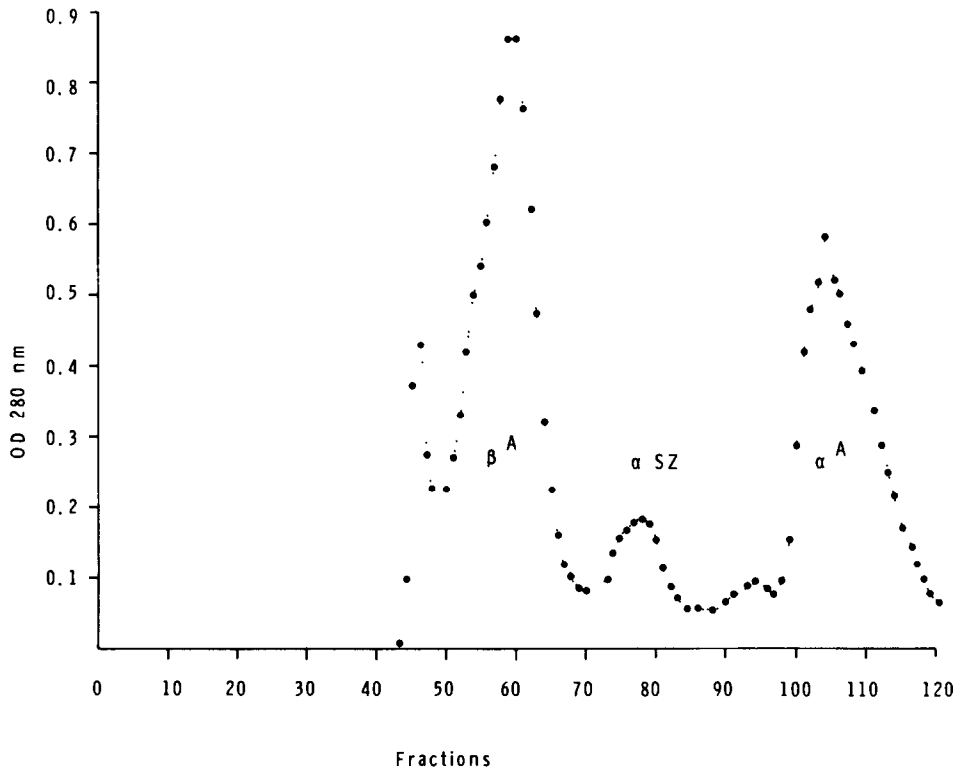

Fig.1. Elution profile of the chains of the globin [3] of the propositus' father.

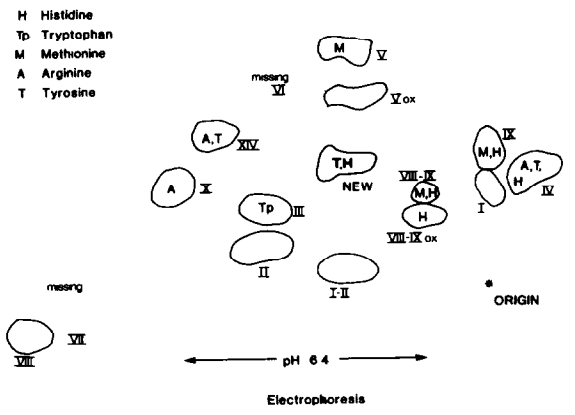

Fig.2. Fingerprint of the soluble peptides of Hb Shaare Zedek. The staining reactions are indicated. Dotted lines show where peptides are missing and the new peptide is shaded. For details see text.

tide was a tryptic peptide, the one Lys found had to be at its C-terminus, and the missing Lys must be that normally found at position α56. This variant, α56 Lys–Glu, has not been described before and is now denoted as Hb Shaare Zedek. The same variant which was seen in the propositus was found in his father. Fig.1 shows a chain separation of the father's globin. The mother was normal although she was slightly deficient in red cell G-6-PD. The propositus showed the male type complete G-6-PD deficiency. Two paternal uncles also had the abnormal haemoglobin band, and so had the father's mother and the propositus' newborn brother. Whilst in the propositus the haemoglobin amounted to 30%, in all others it was about 10% of the total haemoglobin. The father's red cells had some stigmata of α-thalassaemia but the paternal uncles' red cell values were normal.

4. Discussion

The residue α56(E5) is on the surface of the haemo-

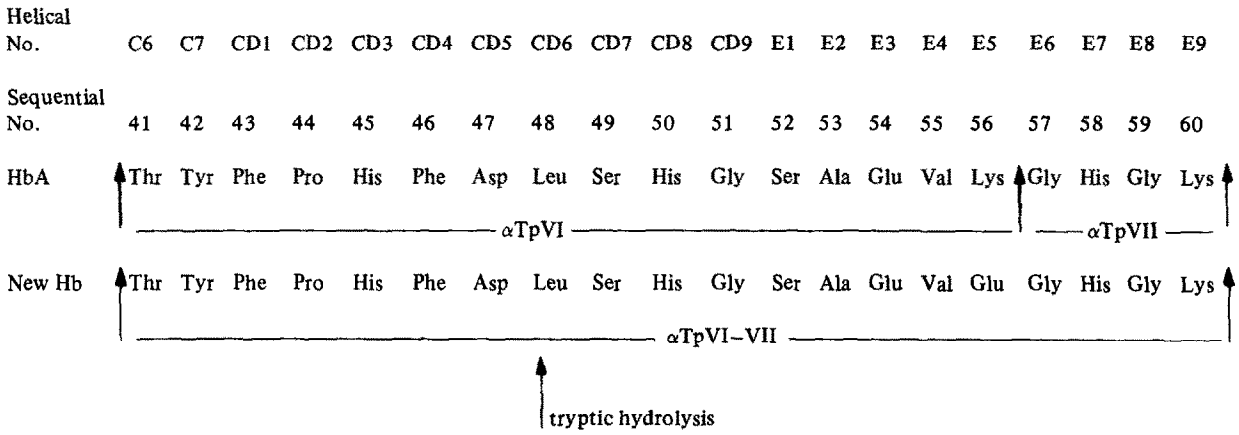

Fig.3. Diagram of amino acid sequences of residues 41–60 of the α-chain. In Hb Shaare Zedek there is no tryptic hydrolysis between residues 56 and 57.

Table 1
Amino acid analysis of the 'new' peptide found in Hb Shaare Zedek

Residue	nmol found	Molar ratio		
		New peptide		TpαVI plus VII (α41–60) (expected)
Asp	5.493	1.10	1	1
Thr	4.383	0.88	1	1
Ser	9.147	1.83	2	2
Glu	9.809	1.96	2	1
Pro	4.779	0.96	1	1
Gly	15.927	3.19	3	3
Ala	5.198	1.04	1	1
Val	3.949	0.79	1	1
Leu	5.435	1.09	1	1
Tyr	4.559	0.91	1	1
Phe	10.078	2.02	2	2
His	15.515	3.10	3	3
Lys	5.714	1.14	1	2

For details see text

globin molecule and is not known to be involved in the maintenance of structure or function of the tetramer. Hb Thailand [4] has its abnormality in the same position as Hb Shaare Zedek but the substitution is Lys → Thr. As in the present case no specific pathological consequences for its carriers were noted. This can also be stated for Hbs with homologous mutations in the β- and γ-chains: N Seattle (βE5 Lys → Glu), Hikari (βE5 Lys → Asn) and F Jamaica ($^{A}\gamma$E5 Lys → Glu) [5–7].

Amongst Iranian and particularly amongst Kurdish Jews, α-thalassaemia and HbH disease are not uncommon. Although the family of the propositus is Persian-speaking, they originated from Kermanshah, which is near Kurdistan. The differences in the proportion of the α-chain abnormal variant seen in this family suggest that α-thalassaemia may well be present in this family also. It is of some practical importance that the demonstration of a HbH-like haemoglobin in a patient with a hypochromic anaemia must, as in this case, be supported by further tests before a diagnosis of HbH disease can be made.

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