HAEMOGLOBIN HANDSWORTH α18 (A16) GLYCINE → ARGININE

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1. Introduction

In the course of a survey of Birmingham schoolchildren [1] a new haemoglobin variant, Hb Handsworth α18 (A16) glycine → arginine was found. The propositus, a 12 year old boy of West Indian origin, showed no clinical abnormalities.

2. Methods

Haematological data were determined using standard techniques. The haemoglobin chains were separated electrophoretically using 'Cellogel' cellulose-acetate strips [2]. Stability was tested by heating and precipitation in isopropanol [3,4]. The proportion of haemoglobin fractions were determined by electrophoresis on cellulose-acetate [5] and the haemoglobin variant was purified by DEAE–Sephadex chromatography [6]. Globin was prepared by precipitation in acid acetone, digested with trypsin and fingerprinted. Peptides containing divalent sulphur, histidine and arginine were located by specific staining reactions [7]. For amino acid analysis peptides were eluted from paper in 6 N HCl and hydrolysed at 105°C for 24 h in sealed capillary tubes [7]. The analyses were obtained with a 'Locarte' amino acid analyser. For N-terminal analysis peptides were eluted from paper in 0.5 M NH_4OH, dried and dansylated by standard techniques [8]. The dansyl derivatives were identified by thin-layer chromatography on polyamide sheets [9].

3. Results

The haemoglobin level was 116 g/litre, but there was no morphological evidence of thalassaemia. No unstable haemoglobin was detected. Electrophoresis of the haemolysate on paper, at pH 8.9, showed HbA and HbA_2, and a band moving slightly slower than, and only just separating from, HbA, in the position of Hb Lepore. A slow HbA_2 was also seen. Electrophoresis of the haemolysate in 6 M urea showed an abnormal α-chain with an increased positive charge.

On the fingerprint of tryptic peptides a new arginine positive spot was located between αTpX (α91–92) and βTpVI (β60–61) (peptide A, fig.1). The amino acid composition of this spot was Val, Arg. As this was a tryptic peptide the sequence must be Val–Arg. This new peptide could have arisen by a point mutation either at residue α2 (Leu) or α18 (Gly), affecting either peptide αTpI (α1–7) or αTpIV (α17–31). The amino acid composition of the spot in the position of αTpIV (α17–31) (peptide B, fig.1) corresponds to residue α19–31 (table 1), indicating that the mutation was at residue α18 (glycine → arginine). No change in electrophoretic or chromatographic mobility would be expected from the loss of residues α17 and 18 from αTpIV (α17–31). N-terminal analysis of the new peptide in the position of αTpIV (α17–31) showed the presence of valine, confirming a new tryptic cleavage at residue α18.

4. Discussion

The mother of the propositus is dead, and neither

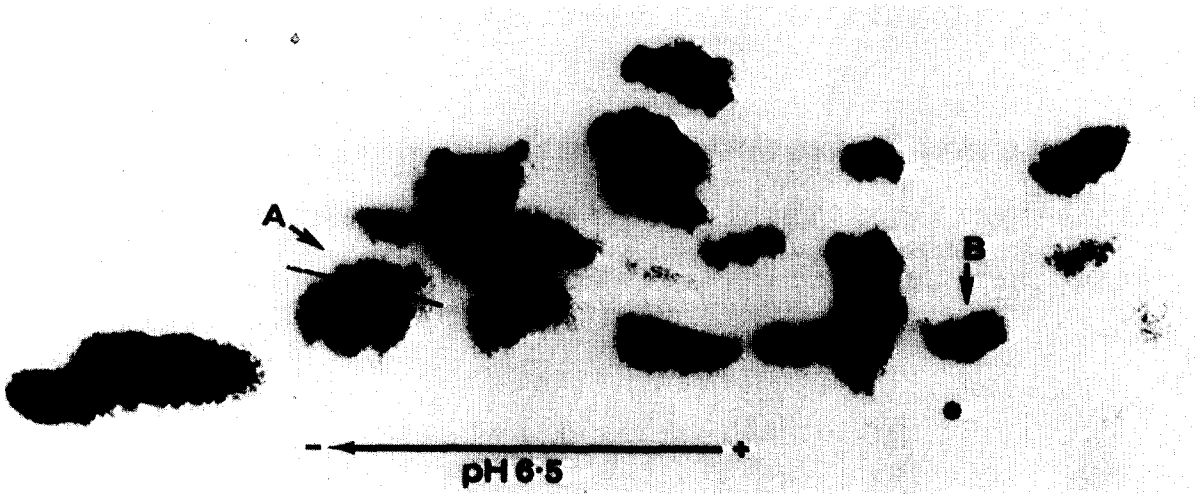

Fig.1. Fingerprint of tryptic peptides of whole globin of Hb Handsworth. A – New arginine positive peptide. B – peptide with N-terminal alanine. (*) lower right, point of application.

Table 1
Amino acid analysis of 'peptide B'

Amino acid	'Peptide B'		αTpIV (α17–31)
Glu	3.1	(3)	3
Gly	2.1	(2)	3
Ala	4.0	(4)	4
Val	0.1[a]	(0)	1
Leu	1.0	(1)	1
Tyr	0.6[b]	(1)	1
His	1.0	(1)	1
Arg	1.0	(1)	1

[a]Possibly some contamination with αTpIV (α17–31)
[b]Some destruction of Tyrosine occurs during acid hydrolysis

Table 2
Proportion of haemoglobin present

Haemoglobin	% Total haemoglobin
HbA_2 (Including variant A_2)	3.1
Hb Handsworth	10.8
HbA	86.1

the father nor any of his other siblings carried the variant. In another family in Birmingham a son from a previous marriage of the deceased mother was found to carry a slow moving α-chain variant amounting to 9% of the total haemoglobin.

The variant is present as only 11% of the total haemoglobin (table 2) and no change in haemoglobin stability was detected. The residue 18 (A16) is the last of the A-helix. It is known that glycine has helix breaking properties and it may be that by substituting an arginine at this point some perturbation of the AB corner occurs, making the mutant α-chains less successful competitors for β-chains and hence the variant is present in a low concentration. The relative importance of this residue is indicated by its conservation in thirteen of the fifteen haemoglobins listed in [10].

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