HAEMOGLOBIN SHERWOOD FOREST β104 (G6) ARG→THR

D. R. RYRIE
Haematology Department, City Hospital, Nottingham NG5 1PD

and

D. PLOWMAN and H. LEHMANN
University Department of Clinical Biochemistry, Addenbrooke's Hospital, Cambridge CB2 2QR England

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1. Introduction

During the routine antenatal screening of a 22-year-old woman of Kashmiri Muslim origin, an abnormal haemoglobin was observed. In the 18th week of pregnancy the haemoglobin level was 10.7 g/dl and the red cells were normocytic and normochromic. Routine medication of folic acid and iron was began, and the haemoglobin level rose to 11.5 g/dl where it remained throughout pregnancy. After an uneventful delivery the haemoglobin level rose to 12.5 g/dl.

2. Methods

Haemoglobin electrophoresis was performed on paper [1] and cellulose acetate [2] at pH 8.9. Stability was tested by heating and precipitation in isopropyl alcohol [3,4]. The haemoglobin chains were separated electrophoretically on 'Cellogel' cellulose acetate strips [5]. Globin was prepared by precipitation in acid acetone (1.5% (v/v) HCl in acetone). Preparative chain separation was carried out on a column of CM cellulose [6]. The abnormal β-chain was aminoethylated [7], freed from excess reagents by passage through a column of Sephadex G-25 (coarse grade), equilibrated with 0.5% (v/v) acetic acid, and recovered by freeze-drying. The purified chain was digested with trypsin and two dimensional peptide maps were prepared [1]. Peptides containing divalent sulphur, histidine, arginine and tyrosine were located by specific staining reactions [1]. Peptides for analysis were eluted in 6 N HCl, containing 0.1% (w/v) phenol and 0.01% (w/v) dithiothreitol, and hydrolysed at 105°C for 24 h in sealed capillary tubes. The analyses were obtained with a 'Locarte' amino acid analyser.

3. Results

Electrophoresis on paper, at pH 8.9, showed Hb A and a band just separating anodally from Hb A. On cellulose acetate electrophoresis, at pH 8.9, the separation was slightly better, but was insufficient to allow quantitation of the abnormal fraction. Electrophoresis in the presence of 6 M urea showed an abnormal β-chain, its position indicating one extra negative charge or the loss of one positive charge per chain. The stability tests were negative. There was insufficient separation of Hb A and Hb Sherwood Forest on cellulose acetate to allow quantitation, but from the preparative chain separation results, the variant appeared to amount to about 50% of the total β-chain.

On the map of the tryptic peptides of the abnormal aminoethylated β-chain a positive divalent sulphur stain was noted in the region of βTpIX (β67–82). Peptides βTpXI (β96–104) and βTpXIIa (β105–112) appeared to be missing (fig.1A, B and C, respectively). The amino acid analysis of the new divalent sulphur staining peptide (table 1) showed it to be similar in composition to βTpXI (β96–104) plus βTpXIIa (β105–112) except that one residue of arginine was

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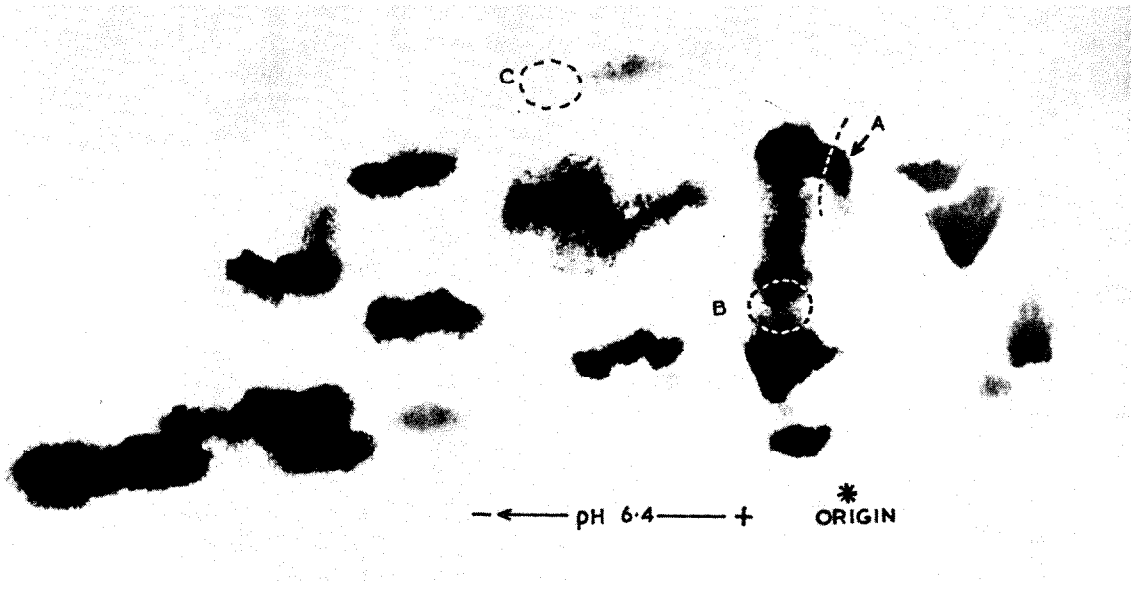

Fig.1. Peptide map of β-chain tryptic peptides of Hb Sherwood Forest. (A) New divalent sulphur staining spot containing peptide β96–112. (B) Position of normal βTpXI (β96–104). (C) Position of normal βTpXIIa (β105–112).

missing and one residue of threonine was present. This indicated a substitution of β104 Arg by Thr. This would account for the absence of peptides βTpXI (β96–104) and βTpXIIa (β105–112), as trypsin hydrolyses bonds only on the C-terminal side of Arg, Lys and aminoethyl-Cys. The substitution of β104 Arg by Thr also accounts for the electrophoretic and chromatographic mobilities of the new peptide (β96–112), and for the behaviour of the abnormal chain on electrophoresis in 6 M urea. The new haemoglobin variant is therefore $\alpha_2\beta_2$ 104 Arg→Thr. The sequence of the normal and abnormal peptides are shown in fig.2.

This is a haemoglobin which has not been described

Table 1
Amino acid analysis of peptide A

Amino acid	nmol	Residues	Residues expected from βTpXI (β96–104) plus βTpXIIa (β105–112)
Asp	18.47	3.14	3
Thr[a]	4.71	0.80	0
Glu	5.94	1.01	1
Pro	5.59	0.95	1
Gly	5.88	1.00	1
Val	17.12	2.91	3
Leu	22.29	3.79	4
Phe	4.71	0.80	1
His	6.12	1.04	1
Cys(AE)[a]	4.12	0.70	1
Arg	0	0	1

[a]Partially destroyed during acid hydrolysis

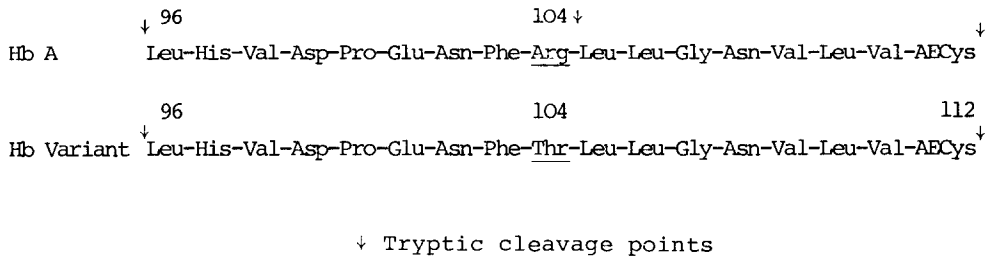

Fig.2. Sequence of normal and abnormal β-chain peptides.

so far and because of the location of the hospital where it was found it has been designated Haemoglobin Sherwood Forest.

4. Discussion

Examination of Perutz's model of the haemoglobin tetramer shows that the two β104 (G6) Arg residues point towards each other and into the hydrophilic interior of the tetramer. They are presumably stabilized by the inclusion of an anion between them. The substitution of Thr at position β104 (G6) would not be expected to have much influence on the stability of the molecule. Marginally decreased stability has been reported for a similar variant, Hb Camperdown $\alpha_2\beta_2$ 104 (G6) Arg→Ser [8], but on re-examination of this variant, no evidence of decreased stability was found (Carrell, R. W., personal communication).

The substitution appears to have no influence on the function of the haemoglobin molecule, and the propositus shows no evidence of any clinical or haematological abnormality that can be related to the presence of the variant.

Acknowledgements

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