HAEMOGLOBIN FERNDOWN (α6 [A4] ASPARTIC ACID → VALINE)

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1. Introduction

A 40-year-old British woman, mother of two children aged 10 and 7 years was treated for iron deficiency anaemia for 2 years (serum iron 5.5 μmol/l; total iron binding capacity 84 μmol/l). Inspite of treatment the anaemia persisted and actually became worse. Thus when she was referred to a neurologist with mild temporal lobe epilepsy her Hb was found to be 7.7 g/dl. Her anaemia only improved following hysterectomy in 1978. At the time when her iron deficiency anaemia did not respond in the expected manner to iron therapy, electrophoresis of the haemoglobin was performed to exclude thalassaemia. There was no thalassaemia but an abnormal band was discovered which turned out to be composed of a not yet described haemoglobin variant.

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 summarized [1]. Quantitative haemoglobin and globin chain separation followed established techniques [2,3].

3. Results

On electrophoresis at pH 8.9 of the haemolysate in addition to the Hb A and Hb A_2 bands, a small band was seen moving closely behind Hb A rather faster than Hb S in a Hb A + S control. There was also a very faint band noted moving behind Hb A_2. The first amounted to 8% of the total haemolysate, and the second to <1%. Hb A_2 and F measured by the usual methods were in normal proportions. On citrate agar electrophoresis the abnormal band separated from Hb A. Tests for unstable haemoglobins gave negative results. Globin chain electrophoresis revealed a 'slow' moving α-chain. The abnormal α-chain was separated from the total globin and 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.1). A new peptide was observed which did not stain for any specific amino acid. The tryptic peptide αI–II (α1–11) was absent. The electrophoretic properties of the haemoglobin had indicated that the abnormal chain differed from the α^A chain by the loss of one negative or the acquisition of one positive charge. It thus seemed that the new peptide had replaced a neutral peptide within the group of overlapping neutral peptides comprising αTpI, IV, IX and XI. The tryptic peptides αIV and αIX, both stain for several specific amino acids whereas the new peptide was negative in these tests. It was therefore likely to be either related to αTpI or to αTpXI. The absence of αTpI–II suggested that the former was the case. The tryptic peptide αI–II (α1–7)–(α8–11) arises from incomplete tryptic separation of the two peptides because the negatively charged α6 Asp adjacent to α7 Lys inhibits the action of trypsin on α7 Lys–α8 Thr (fig.2). The interpretation of the fingerprint thus suggested that the new peptide was related to αTpI, and because the electrophoretic properties of the haemoglobin variant sug-

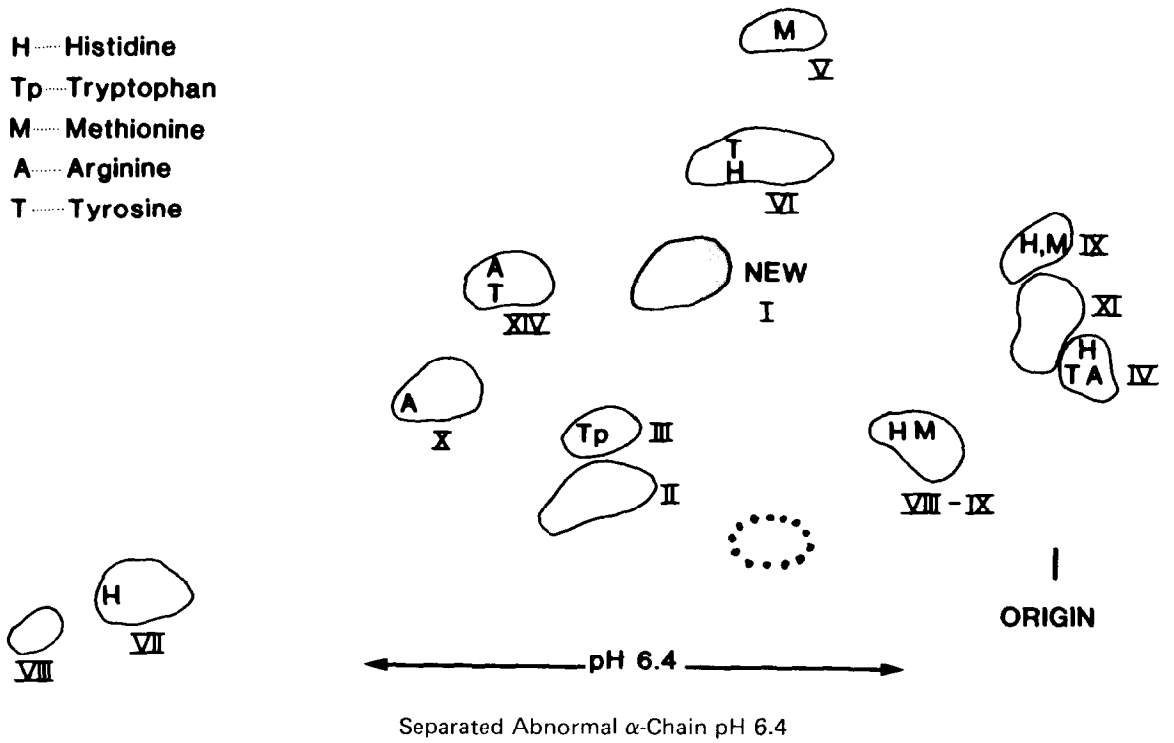

Fig.1. Fingerprint of the tryptic peptides of the α-chain of Hb Ferndown. The dotted line indicates the area where α^{A}Tp1–2 is missing. For details see text.

gested the acquisition of one positive or the loss of one negative charge and because αTpI–II was missing, this difference was likely to arise from a replacement of α6 Asp by a neutral residue.

Amino acid analysis of the new peptide indeed showed that it resembled that expected for αTpI except that the expected residue of aspartic acid was missing and that an additional residue of valine was present instead (table 1). A variant α6 Asp → Val has not been described before and the new haemoglobin was named Ferndown (H. Q. of the Dorset Area Health Authority).

Examination of the patient's parents demonstrated the variant haemoglobin band in the mother. There was again no anaemia and the haematological values were within normal limits (table 2).

4. Discussion

Two haemoglobin variants involving residue α6 Asp have been described: Hb Sawara, Asp → Ala [4] and Hb Dunn, Asp → Asn [5]. Neither is associated with anaemia or with haematological abnor-

Helical No.	NA1	NA2	A1	A2	A3	A4	A5		A6	A7	A8	A9	
Sequential No.	1	2	3	4	5	6	7	↑	8	9	10	11	↑
Hb A	Val^{+}	Leu	Ser	Pro	Ala	ASP^{-}	Lys^{+}		Thr	Asn	Val	Lys^{+}	

In Hb Ferndown Asp is replaced by Val.

Fig.2. A diagram of the amino acid sequence of residues 1–11 of the α^{A}-chain. On tryptic digestion (↑) the peptides αI(α1–7) and αII(α8–11) are formed. With α^{A} the tryptic hydrolysis between residues 7 and 8 is incomplete, because of inhibition by the negatively charged Asp at position 6, and αI–II (α1–11) is also found.

Table 1
Amino acid analysis of the 'new' peptide found in the variant α-chain

Residue	Found (nmol)	Molar ratio	
		New peptide	αTpI (expected)
Asp	1.183	(0.07) –	1
Ser	17.881	(1.10) 1	1
Pro	18.846	(1.16) 1	1
Ala	17.015	(1.05) 1	1
Val[a]	26.280	(1.62) 2	1
Leu	13.718	(0.81) 1	1
Lys	13.421	(0.83) 1	1

[a] One of the two valines is at the N-terminus, and would be partially destroyed by the action of ninhydrin on N-terminal residues

For details see text

malities. The proportion of Hb Dunn is 11% and that of Hb Sawara 21%. Studies of the effect of substituting α6 Asp have been carried out in the case of Hb Sawara [6]. The Bohr effect and cooperativity are the same as for Hb A, but there is a small but definite increase in O_2 affinity, the difference of the log P_{50} at pH 7.0 being 0.37. The α6 Asp forms a salt bridge with α127 (H10)Lys of the same α-chain [7] and from a survey of several dozen human haemoglobin variants with increased O_2 affinity Perutz and Fermi have concluded that any loosening of tertiary structure whether it occurs between the αβ dimers, or within a dimer or within a single subunit, will favour the R structure of the tetramer and raise O_2 affinity [8]. The loss of this salt bridge ($\alpha_1 6$–$\alpha_1 127$) does however not seem to have any pathological consequences, and this is confirmed by the fact that Hb St Claude (α127 Lys → Thr) [9] which like Hb Sawara, Hb Dunn and Hb Ferndown would not be able to form the $\alpha_1 6$–$\alpha_1 127$ salt bridge is also associated with a normal blood picture.

Acknowledgement

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Table 2
Haematological values of the patient and her parents

	Hb Ferndown	Hb A_2 (%)	Hb F (%)	Hb (g/dl)	RBC ($\times 10^{12}$/l)	Hct (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)
Propositus	Present	3.3	0.3	13.7	4.63	39.5	86	29.6	34.4
Father	–	3.2	0.6	15.5	4.98	44.7	89	30.9	34.5
Mother	Present	3.2	0.9	14.1	5.35	42.3	79	26.3	33.3