HAEMOGLOBIN CARIBBEAN β91 (F7) Leu → Arg: A MILDLY UNSTABLE HAEMOGLOBIN WITH A LOW OXYGEN AFFINITY

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1. Introduction

A new mildly unstable haemoglobin, Hb Caribbean, is described, in which the leucine residue β91 (F7), is replaced by arginine. The variant has a lower than normal oxygen affinity.

It was discovered in a West Indian family during the course of an electrophoretic screening programme to detect sickle-cell anaemia in neonates at the Victoria Jubilee Hospital Jamaica. The propositus, an otherwise healthy infant, was found to be a double heterozygote for Hb S and Hb Caribbean.

2. Methods

Haemoglobin electrophoresis was performed on cellulose acetate at alkaline pH [1] and on agar gel at acid pH [2]. Sickling tests were carried out on whole blood [3]; isopropanol stability and heat denaturation tests on haemolysates [4,5]. Haemolysates were fractionated by DEAE-Sephadex chromatography [6] and the abnormal haemoglobin isolated. Globin was prepared from this fraction by precipitation from acid acetone (1.5% (v/v) HCl in acetone) and separated into α and β globin chains by CM-cellulose chromatography [7]. The abnormal β-chain was aminoethylated [8], digested with trypsin and fingerprinted [9]. Peptides containing divalent sulphur, histidine and arginine were located [9]. Peptides were eluted from paper with 6N HCl and hydrolysed at 105°C for 24 h in sealed evacuated tubes containing 0.01% (w/v) dithiothreitol. Amino acid analyses were obtained with a 'Locarte' amino acid analyser.

Oxygen affinity studies on whole haemolysates were performed by the discontinuous spectrophotometric method of Benesch et al. [10] as modified by Bellingham and Huehns [11].

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3. Results

The propositus was a healthy infant with a birth weight of 3750 g. On cellulose acetate electrophoresis, her haemolysate showed no normal adult haemoglobin, but there was a fraction migrating as Hb S. On electrophoresis of their blood, her mother and father both appeared to be heterozygotes for Hb S (Hb A + Hb S). In vitro sickling tests confirmed the presence of Hb S in the propositus and her father, but the mother's red cells behaved normally. On re-examination of the mother's haemolysate by starch gel electrophoresis, the abnormal haemoglobin did not migrate as Hb S but moved to a slightly more anodal position. Agar gel electrophoresis of the propositus's haemolysate, confirmed the presence of an abnormal haemoglobin other than Hb S; she had one fraction which migrated with Hb S and an additional component which was indistinguishable from Hb A.

The mother's haemolysate was subsequently used to isolate and characterise the abnormal haemoglobin, her haematological values are shown in table 1. Iso-

Table 1
Haematological values found on three occasions in the heterozygote for Hb A and Hb Caribbean

		Time after delivery		
		3 days	2 months	8 months
Haemoglobin	g/dℓ	10.3	11.7	9.7
PCV	ℓ/ℓ	0.34	0.36	0.29
MCHC	pg	30	33	33
Red cell count	$\times 10^{12}$/ℓ	6.7	4.08	3.32
MCV	fℓ	93	87	86
MCH	pg	28	29	29
Reticulocytes	$\times 10^{9}$/ℓ	–	50	–
White cell count	$\times 10^{9}$/ℓ	7.0	–	5.0
Serum iron	μmol/ℓ	–	15	21
Total iron-binding capacity	μmol/ℓ	–	64	43
Direct bilirubin	μmol/ℓ	–	3.4	–
Total bilirubin	μmol/ℓ	–	8.6	–

propanol and heat stability tests both indicated mild instability. DEAE Sephadex chromatography resolved the haemolysate into 56% Hb A + Hb F, 39% abnormal haemoglobin and 4% Hb A_2. The amount of Hb F on alkali denaturation was found to be 0.8%. The normal ranges for Hbs F and A_2 are, not more than 0.8% and 2.5–3.5% respectively. Hb A_2 is expected to be raised in carriers of unstable haemoglobins.

Fingerprints of the abnormal aminoethylated β-chain (fig.1) were lacking two peptides, β^{A}TpX (β83–95) and β^{A}TpXa (β83–93) both of which normally react with the reagents for divalent sulphur and histidine. Two new peptides were located; a neutral peptide containing arginine which gave a yellowish-brown colour with ninhydrin, and a positively charged peptide containing histidine and divalent sulphur. The amino acid composition of both new peptides are given in table 2. It is clear from these results that the positively charged peptide has the same composition as the sequence β^{A}92–95 while

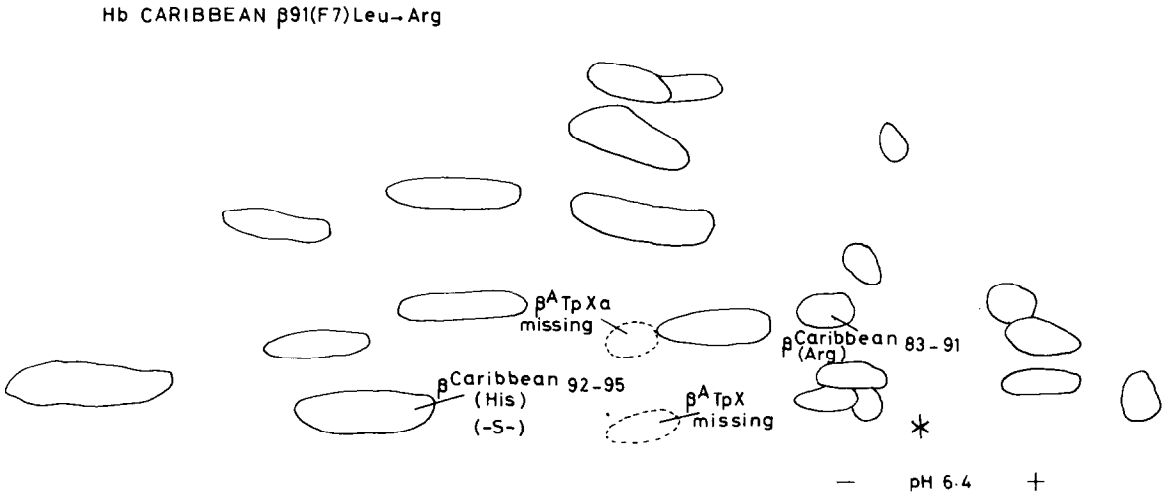

Fig.1. Fingerprint of the tryptic peptides of the amino ethylated β-chain from Hb Caribbean. * = point of application.

Table 2
Hb Caribbean: amino acid composition of the peptides constituting residues β83–95 of the aminoethylated β-chain

Amino Acid	Molar ratios		
	$\beta^{Caribbean}_{83-91}$	$\beta^{Caribbean}_{92-95}$	β^{A}_{83-95} (TpX)
Asp	–	1	1
Thr	1.9		2
Ser	0.9		1
Glu	1.1		1
Gly	0.8		1
Ala	1.1		1
Leu	1.2		2
Phe	1.1		1
His		0.7	1
Lys + AE Cys[a]		1.4	2
Arg	1.0		–

[a] AE Cys, aminoethylcysteine.

the neutral peptide closely corresponds in composition to the sequence β83–91 except that one residue of leucine is missing and there is an additional residue of arginine. From the known specificity of trypsin, arginine must be the C-terminal residue of this peptide and the leucine residue normally found at position β91 has been replaced by a residue of arginine. The variant is, therefore, β91 (F7) Leu-Arg and has been given the name Hb Caribbean.

Hb Caribbean has a lower oxygen affinity than normal. This was shown on whole blood in Kingston and confirmed on haemolysates in Cambridge. The P_{50} value, for normal haemolysates was 2.8 kPa whereas haemolysates from the Hb Caribbean heterozygote gave a P_{50} value of 3.3 kPa for oxygen.

4. Discussion

Leucine β91 (F7), next to the proximal histidine (F8), occupies a position close to the surface of the molecule, but the side chain is directed towards the haem. One of the δ methyl groups comes sufficiently close to make a Van der Waal's contact with a methylene group of the propionic acid side chain on pyrrole ring IV of the haem [12]. Only one variant of human haemoglobin is known at this position, Hb Sabine, β91 (F7) Leu-Pro [14], which causes a severe inclusion body anaemia, as might be expected from the interruption of the α-helix by a residue of the imino acid proline. Replacement of F7 leucine by a residue of arginine, as in Hb Caribbean, however, results in only mild instability of the haemoglobin but a significant lowering of the oxygen affinity. One can only speculate on the changes in conformation which may occur following this substitution: the arginine side chain is most probably accommodated at this position by an orientation of the side chain so that the guanidinium group is at the surface of the molecule. It is highly probable that the haem contact made by F7 leucine is at least partially lost in this variant, and it might be possible for the guanidinium

Residue No.	83	84	85	86	87	88	89	90	91	92	93	94	95
Helical No.	EF7	EF8	F1	F2	F3	F4	F5	F6	F7	F8	F9	FG1	FG2
β^{A}	Gly	Thr	Phe	Ala	Thr	Leu	Ser	Glu	Leu	His	Cys	Asp	Lys
$\beta^{CARIBBEAN}$	Gly	Thr	Phe	Ala	Thr	Leu	Ser	Glu	Arg	His	Cys	Asp	Lys

Fig.2. The amino acid sequence of residues β83–95 of HbA (β^{A}TpX) and Hb Caribbean.

group to interact with the carboxyl group of the propionic acid side chain, which normally forms a salt bridge with the ϵ-NH_2 group of lysine β66 (E10).

Leucine occupies this position in the reported sequences of α-, β- and γ-chains from all known mammalian haemoglobins [13] though the residue F7 is, interestingly, arginine in the β-chain of the bull-frog *(R. catesbeiana)* [15].

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