HAEMOGLOBIN NORTH SHORE-CARACAS β134 (H12) VALINE → GLUTAMIC ACID

T. ARENDS

Instituto Venezolano de Investigaciones Cientificas (IVIC), Departmento de Medicina Experimental, Apartado 1827, Caracas 101, Venezuela

and

H. LEHMANN, D. PLOWMAN and R. STATHOPOULOU

University of Cambridge, Department of Clinical Biochemistry, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 2QR, England

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1. Introduction

A new slightly unstable haemoglobin, Hb North Shore-Caracas β134 (H12) valine to glutamic acid is described. The propositus, who has an English mother and a Venezuelan father, presented with anaemia and recurrent respiratory tract infections. He was found to be a double heterozygote for HbS and Hb North Shore-Caracas. Three more carriers of this haemoglobin were discovered in the family.

On biosynthesis studies, less labelled unstable β globin was present than β^A or β^S globin.

2. Methods

The haematological data were obtained by standard techniques [1]. Serum iron and iron-binding capacity were measured according to Stookely [2]. Oxygen affinity studies were performed on whole haemolysates by the modified discontinuous spectrophotometric method [3,4].

Haemoglobin electrophoresis was performed on paper [5] and cellulose acetate, at pH 8.9 [6]. The haemoglobin chains were separated electrophoretically on 'Cellogel' cellulose acetate strips [7]. Stability was tested by heating and precipitation in isopropyl alcohol [8,9]. The proportions of haemoglobin fractions were determined by cellulose acetate electrophoresis [6]. The abnormal fraction was partially purified by heat precipitation [8]. Globin was prepared by precipitation in acid acetone, and then digested with trypsin. Two-dimensional peptide maps were prepared by electrophoresis and chromatography [5]. Peptides containing divalent sulphur, histidine, arginine and tyrosine were located by specific staining reactions. The abnormal peptide was purified by electrophoresis, at pH 9.0, eluted in 2% (v/v) NH_4OH and dried. The dried peptide was dissolved in 0.5 ml 50 mM NH_4HCO_3, pH 7.8 and digested with 30 μl 1 mg/ml water *Staphylococcus aureus* strain V8 protease (Miles Laboratories Ltd, PO Box 36, Stokes Poges, Slough SL2 4LY, England) for 24 h at 37°C [10]. The resulting peptides were separated by electrophoresis, at pH 6.4. Peptides for amino acid analysis were eluted in 6 N HCl and hydrolysed at 105°C for 24 h in sealed capillary tubes. The analyses were obtained with a 'Locarte' amino acid analyser.

Measurement of the ratio of α chain/non-α chain synthesis was carried out on a reticulocyte-enriched suspension, incubated with 100 μCi [^{3}H]leucine (spec. act. 58 Ci/mmol) for 60 min. The procedure followed that of Lingrel and Borsook [11] with some minor modifications [12]; saline was used for incubation instead of plasma. The measurements of the ratio of α chains to non-α chains followed the procedures described before [12].

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3. Results

The propositus, aged 6 years, presented with recurrent respiratory tract infections. Physical examination showed little abnormality except for pallor of skin and mucosae and a congested oropharynx. Haematological examination showed a mild anaemia (10.4 g/dl) and microcytosis. Electrophoresis of the whole haemolysate on paper, at pH 8.9, showed HbA and HbS, with a slightly raised HbA_2. On cellulose acetate, however, a band just separated anodally from HbA. The abnormal fraction was found to be mildly unstable. The brother, mother and maternal grandmother of the propositus also showed this band. Except for the English grandmother, the other carriers of the new haemoglobin were mildly iron deficient. This is ascribed to a low protein diet. There were no abnormal physical findings, notably no enlargement of the spleen in any of them, but the maternal grandmother had some arthritic complaints. The results of the family study are summarised in table 1.

On the map of the tryptic peptides, histidine staining was noted in the position of αTpI–II (α1–11). The peptide βTpXIV (β133–144) was present in very low yield (fig.1). On electrophoresis, at pH 9.0, the histidine staining spot separated into two. One was normal αTpI–II (α1–11), and the other resembled βTpXIV (β133–144). However, one valine residue was missing and one additional glutamic acid or glutamine residue was present (table 2). Substitution of valine by glutamine cannot arise by a single point mutation, and this suggested a valine to glutamic acid substitution. This was supported by the electrophoretic mobilities of the abnormal haemoglobin and of the abnormal peptide, which indicated an extra negative charge in the variant.

The peptide βTpXIV (β133–144) contains three valine residues, at positions 133, 134 and 137, all of which are internal, non-polar residues. The introduction of the charged glutamic acid residue at any one of these sites could give rise to the observed instability of the haemoglobin. To determine the position of the substitution, the purified peptide was digested with *Staph. aureus* strain V8 protease, which is known

Table 1
Summary of results of family study

	Propositus (Age 6)	Brother (Age 5)	Mother (Age 35)	Maternal grandmother (Age 54)
Hb (g/dl)	10.4	11.2	12.3	11.7
RBC ($\times 10^{12}$/l)	4.4	4.9	5.1	5.0
MCV (fl)	71	68	72	72
MCH (pg)	23.3	22.9	23.8	23.4
MCHC (g/dl)	31.5	32.6	32.0	31.3
Iron (mol/l)	11	10	16	24
G-6-PD	Normal	Normal	Normal	Normal
Unstable Hb	5 min ppt	2 min ppt	5 min ppt	5 min ppt
HbF	1.7%	1.2%	0.8%	0.7%
HbA_2 (normal range 2.5–3.5)	4.9%	4.5%	3.9%	2.9%
Variant Hb (NS-C)	35%			
Electrophoresis paper	? ↑ A_2 + S	? ↑ A_2	? ↑ A_2	No apparent abnormality
Electrophoresis cellulose acetate	HbS + band slightly faster than HbA but slower than HbJ	A + fast band	HbA + fast band	A + fast band
Chain separation				Fast β chain
O_2 affinity P_{50} NR 28.5–31.0 mm Hg pH 7.13	40 mm Hg (5.4 kPa)		30 mm Hg (4.0 kPa)	

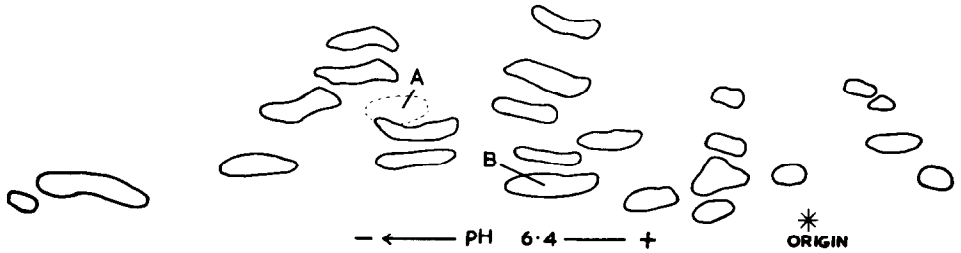

Fig.1. Peptide map of tryptic peptides of Hb North Shore-Caracas. (A) Position of normal βTpXIV (β133–144). (B) New histidine staining spot containing variant βTpXIV (β133–144) and normal αTpI–II (α1–11).

Table 2
Amino acid analysis of the variant TpXIV (β133–144)

Amino acid	Variant βTpXIV (β133–144)		Normal βTpXIV (β133–144)
	Nano moles	Residues	
Asp/Asn	13.3	1.1	1
Glu/Gln	14.3	1.2	0
Gly	14.6	1.3	1
Ala	42.4	3.7	4
Val	21.0	1.8	3
Leu	12.0	1.0	1
His	10.3	0.9	1
Lys	11.8	1.0	1

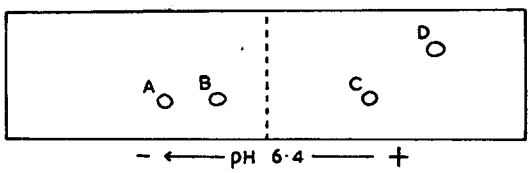

Fig.2. Separation of peptides from *Staph. aureus* strain V8 protease digestion of variant βTpXIV (β133–144). (A) undigested material. (B) β135–144. (C) β133–134. (D) glutamic acid marker.

to split specifically on the C-terminal side of glutamic acid residues [10]. Analysis of the fragments from this digestion showed the presence of a peptide containing glutamic acid and valine only. Its mobility relative to glutamic acid, at pH 6.4, showed it to be a dipeptide (fig.2). A dipeptide Val–Glu could only have arisen by a mutation of β134 valine to glutamic acid (fig.3). The new haemoglobin variant is therefore $\alpha_2\beta_2$134 Val→Glu.

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Normal  ↓- Val - Val - Ala - Gly - Val - Ala - Asn - Ala - Leu - Ala - His - Lys -↓
           133   134   135   136   137   138   139   140   141   142   143   144

Variant ↓- Val - Glu ⇣ Ala - Gly - Val - Ala - Asn - Ala - Leu - Ala - His - Lys -↓
           133   134   135   136   137   138   139   140   141   142   143   144

↓  trypsin cleavage points

⇣  SAV8 protease cleavage points
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Fig.3. Sequence of normal and variant βTpXIV (133–144).

Table 3
Summary of in vitro biosynthesis studies

Case	Total cpm/chain				Ratio	SA cpm/A_{280}				Ratio	Ratio	% Hb^{NS-C}
	α	β^{NS-C}	β^A or	β^S	cpm α/βTotal	α	β^{NS-C}	β^A or	β^S	SA β^{NS-C}/β^A or β^S	cpm β^{NS-C}/β^A or β^S	Lysate
Propositus	21 680	2337		4917	2.98	6038	1249		2026	0.61	0.32	35
Brother	2328	497	870		1.83	1086	462	630		0.73	0.36	34.5
Mother	7314	1783	2527		1.70	1919	747	969		0.77	0.37	36.7

The incorporation of [^{3}H]leucine into the separated α^A,β^A,β^{NS-C} and β^S chains is shown in table 3. It is expressed as cpm ratio of α chains/non-α chains. The propositus, his mother and his brother showed a gross imbalance of globin chain synthesis, the α/non-α ratio being 2.98, 1.70 and 1.83, respectively. These ratios are comparable to our range for β-thalassaemia minor. Sephadex G-100 filtration of the mother's haemolysate showed that the pool of free α chains was greater than normal. The ratio β^{NS-C}/β^A chain is less than 0.50 and this corresponds to the proportion of Hb^{NS-C} in the blood (table 3).

4. Discussion

The residue β134 valine is at an internal site usually occupied by a non-polar side-chain and the substitution of a charged glutamic acid residue at this position will possibly cause some disruption of the tertiary structure. The charge is not fully expressed, as seen from the electrophoretic mobility of the whole haemoglobin. The HbA_2 level is slightly raised, as is commonly seen in cases of unstable β chains. The combination of Hb North Shore-Caracas and HbS results in a mild sickle-cell disease, the proportion of HbS being $> 60\%$.

Whereas the oxygen affinity measured in the heterozygote (mother) is normal, in the case of the association of Hb North Shore-Caracas with HbS, the known decreased oxygen affinity of HbS causes an overall fall of oxygen affinity in the propositus.

The synthesis of the haemoglobin was measured in the reticulocytes which are concerned with only the last 10% of haemoglobin synthesis and may not represent what is happening in the bone marrow. The processing of the washed reticulocytes might have caused a faster denaturation of the unstable haemoglobin North Shore-Caracas, but the results were reproducible in several experiments. The proportion of the synthesised β^{NS-C} chains as determined from the incorporation of the [^{3}H]leucine was diminished. The deficiency of the abnormal chain as well as its inability to form a stable tetramer causes an excess of free α chains. The imbalanced globin synthesis could explain the moderately low haemoglobin level and microcytosis (table 1).

In the propositus, his mother and brother, the serum iron level is low, but it is normal in the maternal grandmother who nevertheless also shows a mild microcytosis.

We understand that an identical haemoglobin has been found in a fourth generation Australian of Anglo-Celtic origin [13] and appears to give essentially the same clinical picture. Its description has been submitted for publication with the name of the haemoglobin given as North Shore. To avoid confusion, we have called our variant Hb North Shore/Caracas.

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