Haemoglobin Korle-Bu (β 73 Aspartic Acid $\rightarrow$ Asparagine)

Showing One of the Two Amino Acid Substitutions of Haemoglobin C Harlem

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In a man aged 65 years whose red cells readily took on the shape of sickle-cells on incubation with 2% sodium metabisulfite, electrophoresis of the haemoglobin on paper (Cradock-Watson, Fenton, and Lehmann, 1959) and starch at pH 8.6 (Poulik, 1957), failed to demonstrate Hb A, and a single band only was seen in the position of Hb S (Fig. 1). Some A₂ was also noted in the normal concentration. This finding is usually associated with sickle-cell anaemia; however, the subject had never suffered from sickle-cell crises or anaemia. He had been a prominent footballer in his younger years. The result of the solubility test of Itano (Itano, 1953) was compatible with sickle-cell trait rather than sickle-cell anaemia. It was, therefore, suspected that the haemoglobin consisted of two different pigments: sickle-cell haemoglobin and another soluble haemoglobin of similar electrophoretic mobility at pH 8.6. This other haemoglobin was unlikely to be Hb D Punjab (or Los Angeles) because Hb S D disease is known to cause clinical symptoms in Accra as elsewhere (Ringelmann *et al.*, 1967). The results of the Itano test also indicated that the proportion of the non-sickling haemoglobin was like that of haemoglobin A in a sickle-cell trait carrier (A + S), rather than that of D in sickle-cell Hb D disease (S + D Punjab).

On agar gel electrophoresis at pH 6 (Robinson *et al.*, 1957), two haemoglobins separated, one in position of Hb S, and one, amounting to two-thirds of the total, in the position where Hb A or D would be found (Fig. 2). These are the findings reported on one occasion in a Nigerian with Hb D Ibadan and S (Watson-Williams *et al.*, 1965). It was, therefore, thought that the present case represented the second case of this unusual type of sickle-cell trait.

Investigations

Identification of Haemoglobin Variant. The variant was separated from Hb S by the same method by which Hb D Ibadan was separated from Hb S (Watson-Williams *et al.*, 1965). As the electrophoretic mobility was so similar to Hb S the usual separation methods could not be applied, but use was made of the low solubility of reduced sickle-cell haemoglobin. The untreated haemolysate was reduced with sodiumhydrosulfite, and using the same phosphate buffer and proportions as described by Itano (1953), the sickle-cell haemoglobin was precipitated and removed by centrifuging. The supernatant was dialysed against water, concentrated *in vacuo*, and the precipitation procedure was repeated to remove any remaining traces of Hb S. The supernatant was again dialysed.

The haemoglobin was then converted into globin, the globin digested with trypsin, and fingerprints of the tryptic peptides were prepared using the established methods (Watson-Williams *et al.*, 1965; Sick *et al.*, 1967). Fig. 3 shows that the tryptic peptide β IX which contains the residues 67–82 of the 146 residues of the β chain was missing. A new peptide was seen next to β TpII, and its position indicated that it contained one more positive charge than β^A TpIX. It stained positive for histidine presumably due to the presence of β 77 histidine (Fig. 4). The peptide was eluted and amino acid analysis of the neutral and acidic residues gave the same results as those expected for β TpIX of Hb A (Table I). During the acid hydrolysis of the peptide which precedes amino acid analysis asparagine is converted to aspartic acid. Although the variant peptide differs on electrophoresis from β^A TpIX by an additional positive charge, it would not differ from β^A TpIX on amino acid analysis if it contained an asparagine where Hb A contains an aspartic acid.

In β^A TpIX two aspartic acid residues are found (Goldstein, Konigsberg, and Hill, 1963): β 73 which is substituted by asparagine in Hb C Harlem (Bookchin, Nagel, and Ranney, 1967), and β 79 which shows this substitution in Hb G Accra (Lehmann, Beale, and Boi-Doku, 1964). To determine the point of substitution in the present case the peptide β TpIX was examined

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FIG. 1. Paper electrophoresis (TRIS buffer, pH 8.9) of the haemoglobin solution of the propositus with Hb A+S as control. A single band is seen in the position of Hb S.

further by chymotryptic digestion. (See Sick *et al.*, 1967.)

Six fingerprints, each of 7 mg. globin, were prepared. They were examined under ultraviolet light when β TpII becomes visible because of its tryptophan content

(β 15). This area was marked with a pencil. The fingerprints were then dipped in dilute ninhydrin (0.02% w/v in acetone), which lightly stained the tryptic peptides and located the aberrant β TpIX which was then cut out, carefully avoiding β TpII. The peptides were eluted with 0.5 M NH_4HCO_3 , and 0.05 ml. chymotrypsin (Worthington 2 mg./ml.) were added, and the digest was incubated for 4 hours at 38°C. After lyophilization, the dried material was redissolved in pH 6.4 buffer for fingerprinting, using electrophoresis for 40 minutes, with a potential gradient of 55 V/cm., and chromatography for 16 hours as for fingerprints of tryptic peptides. Chymotryptic digests of β TpIX from Hb A were prepared at the same time and under the same conditions. In Fig. 4 arrows indicate chymotryptic peptides which could be expected from a complete digestion of β^A TpIX (Sick *et al.*, 1967).

The peptide 67-68 was never obtained, presumably because the initial staining of the tryptic peptide with ninhydrin had altered the N terminal residue. Also 79-81 was not found and only 79-82 was observed. In addition, there was sometimes incomplete cleavage of the leucine bonds at positions 75 and 78, respectively, resulting in peptides of the composition 72-78 and 76-82.

Fig. 5 shows the fingerprint of the chymotryptic digest of the variant β TpIX which was compared with that of β^A TpIX prepared under the same conditions. In preparations from Hb A the chymotryptic peptide 72-75 is acidic because of the aspartic acid residue at position 73 (Sick *et al.*, 1967). This peptide is missing in the fingerprint of the chymotryptic peptides of β TpIX of the variant haemoglobin and a new peptide is present in the neutral area. This was analysed and gave the same

FIG. 2. Agar gel electrophoresis (pH 6) of the haemoglobin solution of the propositus with Hb A+S as control. Two haemoglobins separate, one Hb A as the major fraction, and one moving more slowly—Hb Korle-Bu.

FIG. 3. Fingerprint of Hb Korle-Bu. The arrow indicates the normal position of β TpIX ($\beta 67-82$). A trace is present, presumably due to contamination with β TpIX from the Hb S. A new peptide is seen bordering on β Tp II

FIG. 4. The amino acid sequence of the ninth tryptic peptide of the β -chain of Hb A (β^A TpIX). Arrows indicate where chymotryptic cleavage would be expected.

TABLE I
AMINO ACID COMPOSITION OF
HYDROLYSATE OF ABNORMAL
PEPTIDE IN Hb KORLE-BU AND OF
 β TpIX IN Hb A

	Hb Korle-Bu		Hb A	
	μ mole	Molar Ratio	μ mole	Molar Ratio
Asp	0.117	2.9	0.099	3.0
Ser	0.044	1.1	0.035	1.1
Gly	0.088	2.2	0.069	2.1
Ala	0.087	2.2	0.069	2.1
Val	0.042	1.0	0.032	1.0
Leu	0.150	3.8	0.126	3.9
Phe	0.041	1.0	0.032	1.0
His	0.020	1.0	0.016	1.0
Lys	0.017	1.0	0.014	1.0

Note: Two different preparations of the same peptide were examined, one for neutral and acidic, and one for basic amino acids.

TABLE II
AMINO ACID COMPOSITION OF
CHYMOTRYPTIC PEPTIDE $\beta 72-75$
FROM Hb A AND Hb KORLE-BU

	Hb A		Hb Korle-Bu	
	μ mole	Molar Ratio	μ mole	Molar Ratio
Asp	0.028	1.0	0.012	1.0
Ser	0.028	1.0	0.011	0.9
Gly	0.029	1.0	0.014	1.1
Leu	0.028	1.0	0.014	1.1

composition as the 72-75 peptide from Hb A (Table II). This could only arise if the aspartic acid found at position 73 in Hb A had been replaced by asparagine in the variant. As asparagine is converted to aspartic acid on amino acid analysis, one would expect no difference by this method between the two peptides 72-75, one containing 73 asparagine and one containing 73 aspartic acid. All the other peptides in the fingerprints of Hb A and the new variant were analysed, and all gave the result expected for Hb A. Their position is indicated in Fig. 5.

The conclusion was drawn that the present haemoglobin represents a new variant—Hb Korle-Bu— $\alpha_2 \beta_2$ 73 Asp $\rightarrow$ Asn.

Family Study. After the investigation of Hb Korle-Bu was completed the blood of the man's wife and three of his children became available. The wife is a sickle-cell trait carrier (A + S), and so is one son. As the propositus, the father, has no Hb A, one may conclude that this son inherited the gene for this haemoglobin from his mother and the gene for Hb S from the propositus. One daughter, aged 28, and 24 weeks pregnant without any complications, showed the same haemoglobin combination as the father, i.e. Hb Korle-Bu and Hb S. In her case the gene for Hb S must have been derived from the mother and that for Hb Korle-Bu from the father. Lastly, one son had Hb A and Hb Korle-Bu, presumably the first from his mother and the second from his father. From this limited study one can conclude that the genes for Hb S and Hb Korle-Bu are alleles and that the haemoglobins segregate as Mendelian characters.

FIG. 5. Chymotryptic peptides from β TpIX of Hb Korle-Bu. The broken line indicates where β 72-75 is found normally.

Discussion

Haemoglobin Korle-Bu amounts to more than 50% when present together with Hb S. In that respect it resembles Hb J Baltimore (Charache and Conley, 1964), and Hb D Ibadan (Watson-Williams *et al.*, 1965). There is no haemoglobinopathy and

the carrier of Hb Korle-Bu plus S has the sickle-cell trait rather than a modified sickle-cell disease. One can therefore consider Hb Korle-Bu as an unusual rather than an abnormal haemoglobin.

The additional interest of Hb Korle-Bu, however, is that its mutation is also that of Hb C Harlem

FIG. 6. Homologous crossing over between the genes responsible for Hb S (β 6 Val) and Hb Korle-Bu (β 73 Asn) could give rise to one gene responsible for Hb C Harlem (β 6 Val, β 73 Asn) and one for Hb A (β 6 Glu, β 73 Asp).

(Bookchin *et al.*, 1967). This variant shows in addition a mutation in position $\beta 6$, namely Glu $\rightarrow$ Val, that found in Hb S.

When Hb C Harlem ($\beta 6$ Glu $\rightarrow$ Val and $\beta 73$ Asp $\rightarrow$ Asn) was described, the authors considered that it might have arisen either from a double mutation or from a homologous crossing over in a heterozygote for the genes respectively for Hb S and for a hypothetical haemoglobin $\beta 73$ Asn. Haemoglobin Korle-Bu represents this hypothetical variant. The subject whom we have investigated is exactly the heterozygote envisaged in whom a homologous crossing over would give rise to Hb C Harlem (Fig. 6).

Summary

The haemoglobin of a 65-year-old man whose red cells were found to sickle showed on routine electrophoresis a single major band in the position of Hb S. As the clinical condition was not that of sickle-cell anaemia the haemoglobin was investigated further. Haemoglobin S was found and a new Hb, Korle-Bu ($\beta 73$ aspartic acid $\rightarrow$ asparagine), which formed the major part of the total haemoglobin and which in combination with Hb S did not give rise to sickle-cell disease.

The mutation in Hb Korle-Bu is the same as that found in Hb C Harlem, which in addition shows the sickling mutation at $\beta 6$. This supports the possibility that Hb C Harlem arises from a homologous crossing over of the genes for Hb S and Korle-Bu.

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