A NEW HAEMOGLOBIN J FROM TURKEY – HB ANKARA (β10 (A7) ALA → ASP).

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1. Introduction

During routine investigations on 53-year-old female, admitted to the Department of Internal Medicine at Ankara University with iron-deficiency anaemia, hepatosplenomegaly and cardiac complaints, a fast-moving haemoglobin was noted on starch gel electrophoresis. The J-like haemoglobin was characterised as α_2 β_2 10 (A7) Ala → Asp and was named Hb Ankara.

2. Methods

Haemoglobin (Hb) electrophoresis was performed on paper in Tris buffer at pH 8.9 [1] and on starch gel at pH 8.6 [2]. Haemolysates were fractionated using column chromatography as described by Huisman and Dozy [3]; the resulting haemoglobin fractions were concentrated by ultrafiltration at 4°C and purified by paper electrophoresis [1].

Globin was prepared from purified haemoglobin by acid-acetone precipitation at −20°C [4], washed three times with cold acetone and dried in vacuo over P_2O_5. Digestion of the total globin with trypsin and analysis of that portion of the digest which was soluble at pH 6.4, by fingerprinting, was carried out as previously described [5]. The abnormal peptide from Hb Ankara was isolated by preparative fingerprinting followed by paper electrophoresis at pH 3.5 (55 V/cm for 2hr), located by staining with dilute (0.02%, w/v) ninhydrin in acetone (containing 1% v/v pyridine) and eluted with 0.5 N NH_4OH. For amino acid composition studies, a portion of the eluate was dried and hydrolysed, in a sealed tube, with constant-boiling HCl for 16 hr at 108°C; after removal of excess HCl in a rotary evaporator, the amino acid composition of the dried hydrolysate was determined using a Locarte amino acid analyser [6].

The sequence of the first residues of the variant peptide was determined by dansyl (DNS)–Edman degradation; the Edman procedure was that described by Gray [7] and dansylation was performed according to Brown and Perham [8]. DNS-peptides were hydrolysed for 4 hr at 108°C in 6 N HCl [9] and DNS-amino acids were identified by chromatography on polyamide thin layers [10].

3. Results

On paper electrophoresis at pH 8.9, the patient's haemolysate showed three fractions, namely [1] Hb A [2] a poorly-resolved band moving more rapidly towards the anode, in the position of Hb J and [3] a single Hb A. Column chromatography resolved the

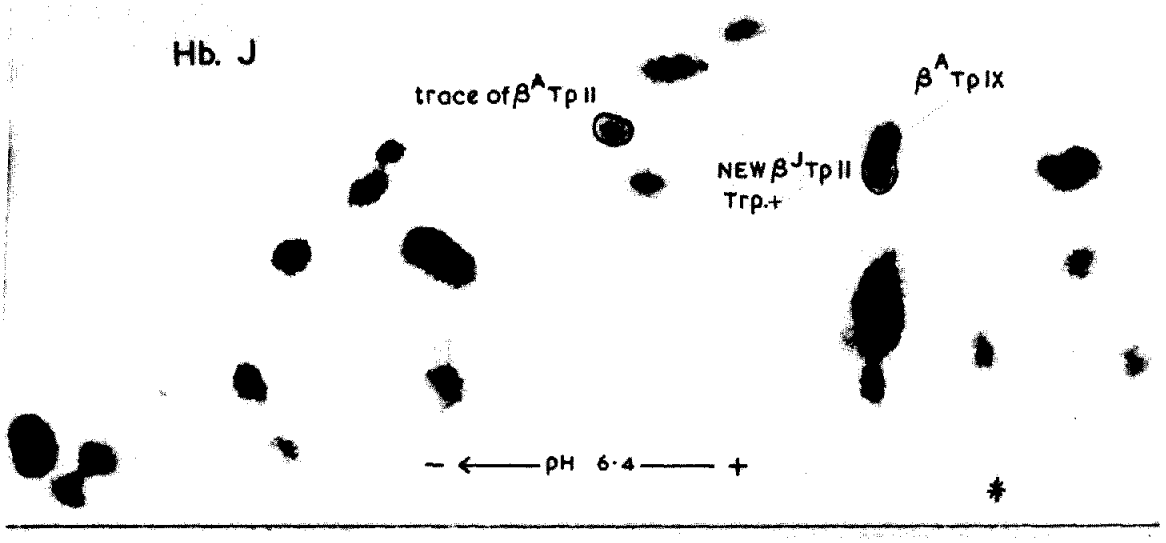

Fig. 1. Fingerprint of the soluble tryptic peptides of Hb Ankara. Electrophoresis at pH 6.4, 53 V/cm for 1 hr. Ascending chromatography in pyridine–isoamyl alcohol–water (6:6:7, by vol.) for 19 hr. Peptides located by staining with 0.2% (w/v) ninhydrin in acetone containing 1% (v/v) pyridine.
* = origin.

haemolysate into 3% Hb A_2, 55% Hb A + A_3 and 42% Hb J + J_3. Even after further purification by paper electrophoresis the Hb J fraction was seen, on fingerprinting, to still contain some Hb A_3.

Comparison of the fingerprint obtained from the variant haemoglobin with the pattern produced by Hb A (fig. 1) indicated that the positively-charged peptide βTpII (residues β9–17), which normally gives a staining reaction for tryptophan, was very much reduced in amount and that there was present a new tryptophanpositive peptide, overlapping β^ATpIX, in the neutral region. Both peptides gave a transient brown colour on staining with ninhydrin, indicative of N-terminal serine; the β^ATpII present on the fingerprint was probably derived from Hb A_3.

Amino acid analysis of the variant peptide showed its amino acid composition (table 1) to resemble that of β^ATpII except that one of the two residues of alanine (β10 or β13) normally found in this peptide (see fig. 2) had been replaced by a residue of aspartic acid. Asparagine also appears as aspartic acid on amino acid analysis after acid hydrolysis, but an alanine to asparagine substitution, which in any case could not arise from a single point mutation, could be eliminated on the basis of the electrophoretic mobility of both the variant haemoglobin and peptide. Dansyl–Edman degradation of the peptide β^JTpII showed that alanine β10 had been replaced by aspartic acid, making it clear

Table 1
Amino acid composition of peptide βTpII from Hb Ankara

Amino acid	Hb Ankara	Expected for Hb A
Asp	*0.9*	–
Thr	1.0	1
Ser	1.0	1
Gly	1.0	1
Ala	*1.1*	2
Val	1.0	1
Leu	1.1	1
Lys	1.0	1
Trp	+	+

One amino acid residue is approximately 31 nmoles.
+ Indicates a positive staining reaction for tryptophan (which is destroyed during the hydrolysis with HCl preceding amino acid analysis).

Residue No.	9	10	11	12	13	14	15	16	17
Helical No. Residues	A6	A7	A8	A9	A10	A11	A12	A13	A14
Hb A	Ser	Ala	Val	Thr	Ala	Leu	Trp	Gly	Lys
Hb Ankara	Ser	ASP	Val	Thr	Ala	Leu	Trp	Gly	Lys

Fig. 2. Amino acid sequence of the tryptic peptide βTpII from Haemoglobins A and Ankara

that the substitution in Hb Ankara is β10 (A7) Ala → Asp, a mutation which has not been reported previously.

Generally speaking, substitution of amino acids at sites which are external in the haemoglobin quaternary structure do not grossly affect the properties of the tetramer [11]. The substitution in Hb Ankara is at an external site, which therefore plays no part in subunit contacts or haem binding; in view of the fact that the relative proportion of the variant haemoglobin was approximately that for a normal, stable β-chain variant, it seems unlikely that the presence of Hb Ankara in the erythrocytes of this patient would have contributed to her anaemia. Unfortunately, no detailed clinical and haematological data were available to assess the likely cause of anaemia and none of the patient's family was examined.

References

[1] Cradock-Watson, J. E., Fenton, J. C. B. and Lehmann, H. (1959) J. Clin. Pathol. 12, 372–373.
[2] Poulik, M. D. (1957) Nature 180, 1477–1479.
[3] Huisman, T. H. J. and Dozy, A. M. (1965) J. Chromatog. 19, 160–169.
[4] Winterhalter, K. H. and Huehns, E. R. (1964) J. Biol. Chem. 239, 3699–3705.
[5] Sick, K., Beale, D., Irvine, D., Lehmann, H., Goodall, P. T. and MacDougall, S. (1967) Biochim. Biophys. Acta 140, 231–242.
[6] Spackman, D. H., Stein, W. H. and Moore, S. (1958) Anal. Chem. 30, 1190–1206.
[7] Gray, W. R. (1967) Meth. Enzymol. 11, 469–475.
[8] Brown, J. P. and Perham, R. N. (1973) Eur. J. Biochem. 39, 69–73.
[9] Gross, C. and Labouesse, B. (1969) Eur. J. Biochem. 7, 463–470.
[10] Hartley, B. S. (1970) Biochem. J. 119, 805–822.
[11] Perutz, M. F. and Lehmann, H. (1968) Nature 219, 902–909.