STRUCTURAL AND FUNCTIONAL CHARACTERISTICS OF Hb TÜBINGEN: β 106 (G 8) Leu → Gln

E. KOHNE, H. P. KLEY and E. KLEIHAUER
Department of Pediatrics, University of Ulm, D-7900 Ulm, Prittwitzstr. 43, West Germany

H. VERSMOLD
Department of Pediatrics, University of Munich, D-8000 München 2, Lindwurmstr. 4, West Germany

H. Ch. BENÖHR
Department of Internal Medicine, University of Tübingen, D-7400 Tübingen, Otfried-Müller-Str. 10, West Germany

G. BRAUNITZER
Max Planck Institute, D-8033 Martinsried bei München, Am Klopferspitz, West Germany

Received 19 March 1976

1. Introduction

Haemoglobin Tübingen was discovered by Kleihauer and co-workers [1] in two members of a German family. The carriers suffered from a variable, compensated haemolysis and a cyanosis due to methaemoglobinemia, methaemoglobin being elevated to 8–12%. The abnormal haemoglobin was characterized by heat instability, absence of spontaneous Heinz body formation, increased spontaneous oxidation and normal methaemoglobin spectra. Preliminary globin analyses suggested a defect in the core of the β chain.

In this paper we present data on the amino acid sequence of the abnormal peptide and the functional characteristics of this new variant. The clinical and haematological data of the propositus along with some biochemical studies of Hb Tübingen have been described previously [1].

2. Materials and methods

The studies were performed on freshly drawn venous blood samples, collected in heparin or ACD solution as anticoagulant. Oxygen equilibrium parameters and red cell 2,3-diphosphoglycerate were assayed as described by Versmold et al. [2]. Erythrocytes were washed with isotonic saline, haemolyzed with water and extracted in carbontetrachloride. The abnormal haemoglobin was isolated in the methaemoglobin form by preparative column chromatography on DEAE-Sephadex A 50 according to Huisman et al. [3], using Tris-HCl buffers, containing 100 mg of $K_3Fe(CN)_6$ in 1 litre buffer. Globin was prepared from the isolated abnormal haemoglobin according to Anson and Mirsky [4]. Polypeptide chain separation was achieved on carboxymethylcellulose CM 52 with an ionic gradient and 8 M urea [5].

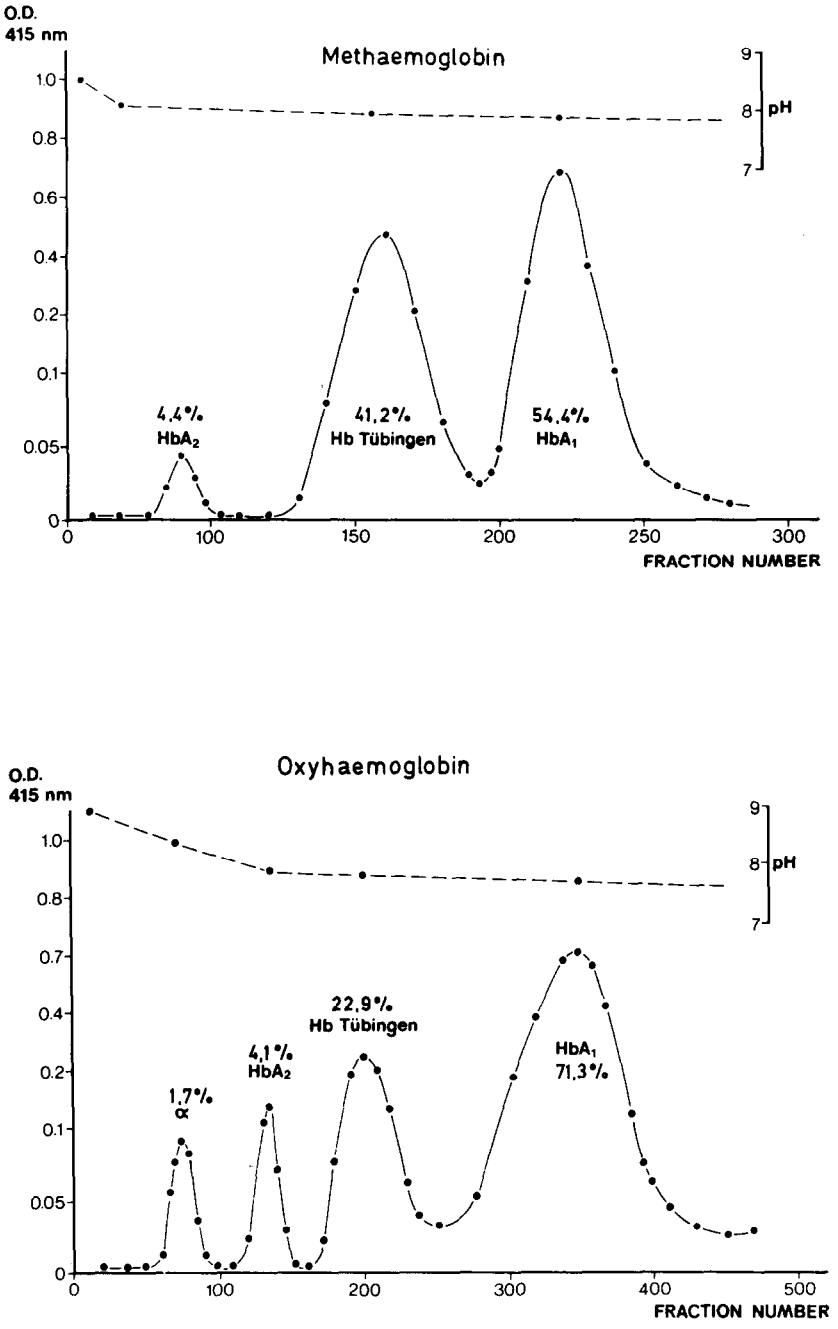

Fig.1. Separation of hemoglobin components from methaemoglobin (upper) and oxyhaemoglobin (lower) haemolysates on DEAE-Sephadex A 50 columns with Tris-HCl buffers. For details see text.

The purified abnormal β chain-*S*-aminoethyl derivative was digested with trypsin [6]. Fingerprints were performed on thin-layer silica gel plates (Merck AG, Darmstadt), using pyridine acetate buffer pH 6.4 for electrophoresis and butanol–pyridine–acetic acid–water (340:200:70:125 by vol.) for chromatography. For preparative purposes the peptides were separated on Dowex 50 W X 2 columns (size:1 X 55 cm), using a linear gradient of pyridine–acetic acid [6].

The peptide-containing fractions from the effluent were identified by reacting an aliquot with fluorescamine (Fluram, Hoffmann La Roche, Grenzach) according to Udenfried et al. [7]. Amino acid analyses were done by a LKB-automated amino acid analyzer BC 200 equipped with high sensitivity cuvettes. Automatic sequence determination was carried out in a Beckman sequencer (Beckman instruments, Palo Alto, California) according to Edman and Begg [8,9] with the modification of the method by Braunitzer et al. [10]. The identification of PTH-amino acids was carried out on silica gel plates 254 as described by Braunitzer et al. [10].

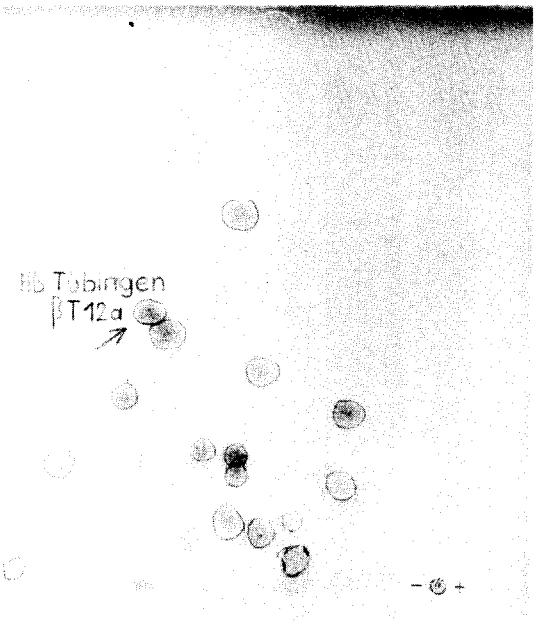

Fig.2. Fingerprint of tryptic peptides from the AE β chain of Hb Tübingen.

3. Results

3.1. *Functional studies*

The oxygen affinity of whole blood containing 8 to 10% methaemoglobin was found to be slightly increased: the partial O_2 pressure for the half saturation of haemoglobin (P 50) was diminished to 21.3 Torr as compared to 27 ± 0.5 Torr in normal subjects. Hill's n_H value was decreased to 2.15. The Bohr effect factor was calculated to be normal (= 0.42). Red cell 2,3-diphosphoglycerate concentration in whole blood was 4.3 μmol/ml erythrocytes (normal = 4.5 ± 0.2 μmol/ml).

3.2. *Chromatography*

The abnormal haemoglobin was completely separated from haemoglobin A_1 only in the methaemoglobin form (fig.1). The amount of Hb Tübingen was 41.2% of total haemoglobin, while it was 22.9%, when oxyhaemoglobin hemolysate was used for chromatographic separation. Free α-chains were elevated to 1.7%.

3.3. *Structural analyses*

Fingerprints of aminoethylated tryptic digested abnormal β-chains showed an altered mobility of the peptide β T 12a (fig.2).

The preparative chromatography of AE β Tübingen chain on Dowex 50 W X 2 revealed (fig.3) an elution pattern similar to the normal β chain except for β T 12a. This peptide emerged earlier in the elution chromatogram than the corresponding normal peptide (hatched zone in fig.3).

The amino acid composition of the β T 12a peptide from Hb Tübingen is listed in table 1, indicating, that one leucine is substituted by a glutamic acid or a glutamine residue.

Automatic Edman degradation followed by identification of the PTH-amino acids on thin layer chromatography showed clearly, that leucine in position β 106 was replaced by glutamine. Thus, the structure of Hb Tübingen is $\alpha_2\beta_2$ 106 (G 8) Leu → Gln. The results of the sequence analysis are given in table 2.

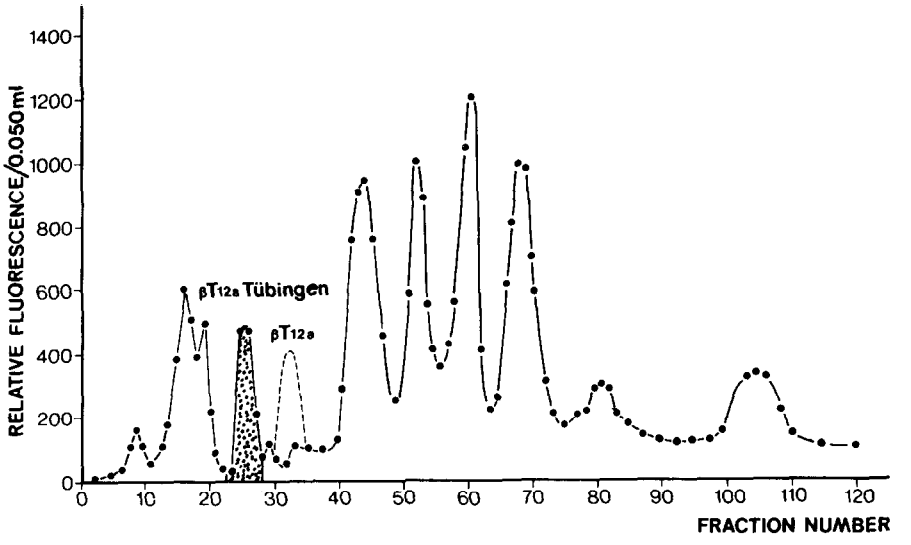

Fig.3. Chromatographic separation on Dowex W-X 2 of tryptic peptides from the aminoethylated β chain from Hb Tübingen. The shaded zone contains the abnormal peptide.

Table 1
Amino acid composition of the β T 12a peptide from Hb Tübingen and HbA

Amino acid	Hb Tübingen β T 12a	HbA β T 12a Found	Expected
AE Cysteine	0.92	0.98	1
Aspartic acid	0.95	0.90	1
Glycine	1.04	0.98	1
Valine	2.25	2.01	2
Leucine	1.87	1.98	3
Glutamic acid (Glutamine?)	1.12	–	–

Results are given as molar ratios.

4. Discussion

Biochemical and structural studies demonstrate that Hb Tübingen is a new variant, characterized by some unique properties, such as different electrophoretic and chromatographic behaviour between the oxyhaemoglobin and the methaemoglobin forms, increased heat instability, slightly increased oxygen affinity and enhanced spontaneous oxidation. Most of these features including the elevated amount of free α chains place Hb Tübingen into the group of the unstable haemoglobin variants.

Absence of spontaneous Heinz body formation and mesobilifuscinuria, two characteristics of unstable

Table 2
Amino acid sequence of the peptide β T 12a of Hb Tübingen

Helical position (G-Helix)	7	8	9	10	11	12	13	14
Sequential No.	105	106	107	108	109	110	111	112
HbA	Leu	Leu	Gly	Asn	Val	Leu	Val	Cys
Hb Tübingen	Leu	Gln	Gly	Asn	Val	Leu	Val	Cys

variants could be explained by the 'pitting' action of the spleen. In helix G the leucine at position 8 is an important residue for the stability of the haemoglobin molecule. As a nonpolar amino acid it occupies an interior position with contacts to the haem group [11]. In haemoglobin Casper [12,13] and haemoglobin Southampton [14] respectively the substitution of leucine at G 8 by proline causes drastic changes in the stability of the molecule, leading to severe haemolytic Heinz body anemia.

However, the substitution of β 106 (G 8) leucine by glutamine, as found in Hb Tübingen, does not disturb the helical formation, because glutamine is, in contrast to proline, a helical forming amino acid. Since glutamine is a polar, water attracting amino acid, it affects the tertiary structure of the molecule and hence its quaternary structure.

Furthermore, the substitution possibly impairs the normal haem contacts at G 8. These molecular alterations may serve as an explanation for the instability, increased oxygen affinity and enhanced autoxidation of haemoglobin Tübingen.

The degree of functional and structural alteration is considerably less. This is in good agreement with the main clinical symptoms of the patient, i.e. moderate haemolysis and methaemoglobinemia.

Acknowledgement

This work was supported by the Deutsche Forschungsgemeinschaft (Kl 169/6 und SFB 51).

References

[1] Kleihauer, E., Waller, H. D., Benöhr, H. Chr., Kohne, E. and Gelinsky, P. (1971) Klin. Wochenschr. 48, 651–658.
[2] Versmold, H., Seiffert, S. and Riegel, K. (1973) Respir. Physiol. 18, 14–25.
[3] Huisman, T. H. J. and Dozy, A. M. (1965) J. Chromat. 19, 160–169.
[4] Anson, M. L. and Mirsky, A. E. (1930) J. Gen. Physiol. 13, 469–476.
[5] Clegg, J. B., Naughton, M. A. and Weatherall, D. J. (1966) J. Mol. Biol. 19, 91–108.
[6] Jones, R. T. (1964) Cold Spring Harbor Symp. Quant. Biol. 29, 297–308.
[7] Udenfried, S., Stein, S., Böhlen, S., Dairman, S., Leimgruber, W. and Weigele, M. (1972) Science 178, 871–872.
[8] Edman, P. and Begg, G. (1967) Eur. J. Biochem. 1, 80–82.
[9] Edman, P. (1970) in: A protein sequenator. (Needleman, S. B. ed.) pp. 211–255, Springer-Verlag, Berlin.
[10] Braunitzer, G., Chen,R., Schwank, B. and Stangl, A. (1973) Hoppe-Seyler's Z. Physiol. Chem. 354, 867–878.
[11] Perutz, M. F. and Lehmann, H. (1968) Nature (London) 219, 902–909.
[12] Jones, R. T., Koler, R. D., Duerst, M. and Stocklen, Z. 2nd International Conference on Red Cell Metabolism and Function. Advances in Experimental Medicine and Biology, Vol. 28, Hemoglobin and Red Cell Structure and Function (Brewer, G. ed.) New York, Plenum Press 1972, p. 79.
[13] Koler, R. D., Jones, R. T., Biglex, R. H., Litt, A., Lovrien, E., Brooks, R., Lahey, M. and Fowler, R.: (1973) Am. J. Med. 55, 549–558.
[14] Hyde, R. D., Hall, M. D., Wiltshire, B. G. and Lehmann, H. (1972) Lancet II, 1170–1172.