Hb STRASBOURG $\alpha_2\beta_2$ 20 (B2) VAL → ASP: A VARIANT AT THE SAME LOCUS AS Hb OLYMPIA β20 VAL → MET

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1. Introduction

The present report describes a new, slightly unstable hemoglobin which has been observed in a Portuguese woman. This new variant, identified as β20 (B2) Val → Asp, is a homologue of Hb Olympia [1] β20 (B2) Val → Met a variant which exhibits a high oxygen affinity and erythrocytosis. In contrast to Hb Olympia, Hb Strasbourg is not associated with erythrocytosis. The new variant of hemoglobin cannot be separated from HbA by routine electrophoretic methods, suggesting that the charge on the aspartic acid side-chain is suppressed as a result of its burial in a hydrophobic region of the molecule. The formation of a salt bridge between the aspartic side chain and an amino group located in the neighbourhood also appears likely.

2. Materials and methods

Routine hematological examinations were performed by standard methods [2] on fresh blood specimens. Hemolysates were prepared according to Drabkin [3]. Tests for heat stability, isopropanol solubility and dissociation resulting from the reaction of hemoglobin with PHMB (*p*-hydroxy-mercuribenzoate) were carried out as previously described [4]. Hemolysates were subjected to electrophoresis on cellulose-acetate [5]. Isoeletric focusing on horizontal polyacrylamide gel [6] was performed over the pH range 6.0–8.0. Globin electrophoresis was carried out in 6.0 M urea [7].

Since the new variant was not separated from HbA by routine chromatographic methods, globin was prepared from the total hemolysate [8]. The abnormal β-chain was separated by carboxymethylcellulose chromatography in 8.0 M urea buffer [9] and aminoethylated. Tryptic peptides were isolated by analytical and preparative fingerprints on thin-layer silica-gel plates (Schleicher and Schüll) according to Braconnier [10] and their amino acid composition established on a Jeol JLC 5 A H amino acid analyser. Sequential analysis of amino acid residues was performed by combined Edman degradation and dansylation as previously described [11].

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3. Results

3.1. *Hematology and hemoglobin electrophoresis*

The new variant was observed in an 18 year old woman who originated from Esposende (northern Portugal). Throughout this time, her hematological

characteristics including red and white blood cell count, platelet count, reticulocyte index, and red cell morphology were completely normal.

Electrophoresis of the propositus sample on cellulose-acetate in Tris–EDTA–borate buffer, at pH 8.6, revealed a wider band than that of the control HbA and resembled that produced by a mixture of HbA and Hb Hope (fig.1). The level of HbA_2 was normal; the isopropanol stability test produced a small precipitate after 20 min. Reaction of hemoglobin with PHMB did not demonstrate the presence of a PHMB unstable variant. Globin electrophoresis in 6.0 M urea indicated the presence of fast moving (β^J)-chains which represented about 40% of the total β-chains. All these results were in accordance with the results obtained with Hb Hope [12]. In fact electrofocusing demonstrated that the hemolysate from the patient did not contain Hb Hope but a variant exhibiting a slightly more acidic p*I* (fig.2).

3.2. *Structural studies*

The fingerprint of the tryptic digest of the aminoethylated β-chain from Hb Strasbourg that the βT3 was replaced by a new, more polar and more negatively charged peptide (fig.3).

The amino acid composition of this new peptide

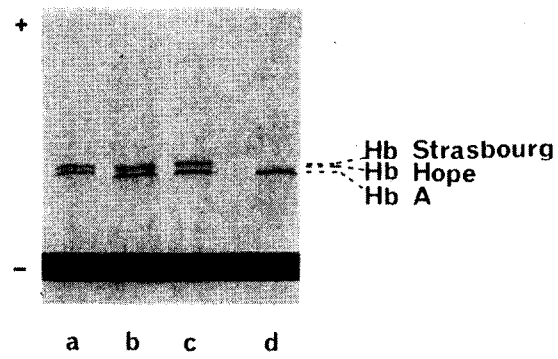

Fig.2. Relative mobilities of two hemoglobin mutants by horizontal polyacrylamide gel isoelectric focusing (left to right): (a) Hope hemolysate, (b) equal mixture of Hope hemolysate and Strasbourg hemolysate, (c) Strasbourg hemolysate, (d) control.

(table 1) differed from normal in lacking one residue of valine and in containing an extra residue of aspartic acid. Asparagine is converted to aspartic acid during hydrolysis which precedes amino acid analysis; a Val → Asn substitution could however be excluded in view of the increased negative charge of the abnormal β-chains.

On account of the presence of three residues of valine in the normal βT3, it was not possible to

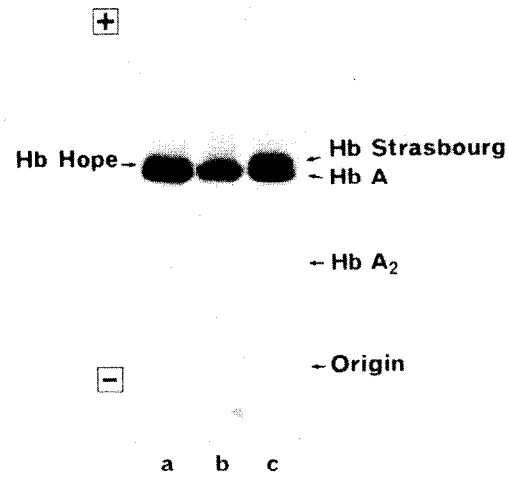

Fig.1. Electrophoresis on cellulose-acetate strips (Tris–EDTA–borate buffer, pH 8.6) of hemolysates prepared from the following (left to right): (a) Hope trait, (b) normal adult, (c) blood from the proband.

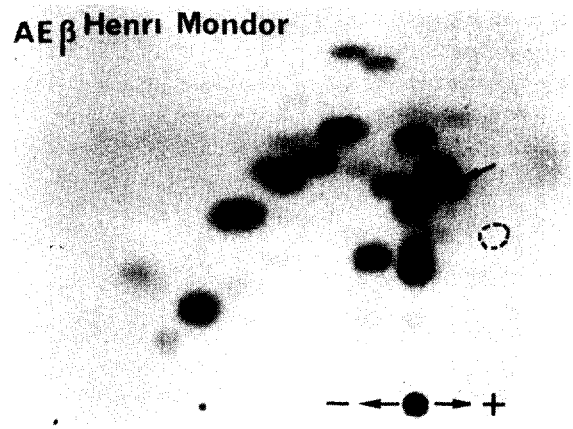

Fig.3. Fingerprint of Tryptic peptides from the AE β-chain of Hb Strasbourg. The arrow shows the position of the new βT3 peptide. The dotted circle indicates the position occupied by βT3 peptide in the control.

Table 1
Amino acid composition of the βT3 peptide from Hb Strasbourg

Amino acid	Molar ratios	
	Found	Normal βT3
Arginine	0.98	1
Aspartic acid	2.98	2
Glutamic acid	2.19	2
Glycine	3.20	3
Alanine	1.02	1
Valine	1.58	3
Leucine	0.99	1

determine which one was replaced by an aspartic acid residue. In order to distinguish between these three positions, the abnormal peptide was eluted from two preparative fingerprints and subjected to dansyl-Edman degradation. The results obtained gave the following sequence:

$$\underset{\text{Val}}{18} - \underset{\text{Asn}}{19} - \underset{\text{Asp}}{20} - \underset{\text{Asp}}{21} - \underset{\text{Glu}}{22} -$$

Hemoglobin Strasbourg therefore contains the amino acid substitution β20 (B2) Val → Asp.

4. Discussion

The new variant, Hb Strasbourg β20 (B2) Val → Asp, is a homologue of Hb Olympia β20 (B2) Val → Met [1], an electrophoretically silent variant which was detected as a result of its high oxygen affinity and associated erythrocytosis. The structural change involves a residue which appears to be located at the surface of the polypeptide and which is not implicated in the allosteric interactions between subunits. Further studies of Hb Strasbourg will be necessary to determine the functional implication of the substitution. It was of interest to compare such results with those obtained for Hb Olympia. Thus no erythrocytosis and no hematological abnormalities could be detected in the patient with Hb Strasbourg.

Since the variant is poorly separated from HbA upon electrophoresis, it seems likely that the charge on the aspartic acid side chain in Hb Strasbourg is buried whilst it is in position within the tetramer.

Another variant of hemoglobin, Hb Hope β136 (H14) Gly → Asp, cannot be separated from HbA by routine electrophoretic methods [12]. Perutz in 1968 [13] proposed the formation of a new salt bridge between Asp 136 and the N-terminal valine in the Hb Hope chain. This hypothesis was recently confirmed by X-ray analyses [14].

Examination of the tertiary structure of the β-chain indicates that the aspartic acid side-chain could probably form a salt bridge with the ε amino group of lysine β65. In Hb J Nyanza α21 (B2) Ala → Asp, which is the homologue on the α-chain of Hb Strasbourg [15], the charge of the aspartic acid residue is not suppressed. Examination of the tertiary structure of the α-chain showed that the twenty-first residue is near the α59 residue which is a glycine; consequently the charge of the aspartic acid could not be suppressed.

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References

[1] Stamatoyannopoulos, G., Nute, P. E., Adamson, J. W., Bellingham, A. J. and Funk, D. (1973) J. Clin. Invest. 52, 342–349.
[2] Dacie, J. V. and Lewis, S. M. (1966) in: Practical Haematology, Vol. IV. (Churchill, ed).
[3] Drabkin, D. L. (1949) Arch. Biochem. 21, 224–231.
[4] Carrell, R. W. and Kay, R. (1972) Brit. J. Haemat. 23, 615–619.
[5] Beuzard, Y., Courvalin, J. C., Cohen-Solal, M., Garel, M. C., Rosa, J., Gibaud, A. and Brizard, C. (1972) FEBS Lett. 27, 76–80.
[6] Monte, M., Beuzard, Y. and Rosa, J. (1976) Amer. J. Clin. Path. in press.
[7] Schneider, R. G. (1974) Clin. Chem. 20, 9, 1111–1115.

[8] Rossi-Fanelli, A. R., Antonini, E. and Caputo, A. (1958) Biochim. Biophys. Acta 30, 608–615.
[9] Clegg, J. B., Naughton, M. A. and Weatherall, D. J. (1966) J. Mol. Biol. 19, 91–108.
[10] Braconnier, F., Beuzard, Y., El Gammal, H., Coquelet, M. T. and Rosa, J. (1975) Nouv. Rev. Fr. Hématol. 15, 527–538.
[11] Weiner, A. M., Platt, T. and Weber, K. (1972) J. Biol. Chem. 247, 3242–3251.
[12] Thillet, J., Caburi, J., Brun, B., Cohen-Solal, M., Garel, M. C., N'Go Minh, M. and Rosa, J. (1974) FEBS Lett. 47, 47–52.
[13] Perutz, M. F. and Lehmann, H. (1968) Nature 219, 902.
[14] Battison, D., Dintzis, H. M. and Perutz, M. F. (in preparation).
[15] Kendall, A. G., Barr, R. D., Lang, A. and Lehmann, H. (1973) Biochim. Biophys. Acta 310, 357–359.