HEMOGLOBIN SAINT MANDÉ β102 (G4) Asn → Tyr: A NEW LOW OXYGEN AFFINITY VARIANT

Nicole AROUS, François BRACONNIER, Joëlle THILLET, Yves BLOUQUIT, Frédéric GALACTEROS, Michel CHEVRIER*, Claude BORDAHANDY* and Jean ROSA

Unité de Recherches sur les Anémies, INSERM U.91, Laboratoire de Biochimie, Hôpital Henri Mondor, 94010 Créteil and **Laboratoire de Biochimie et d'Immunochimie, Hôpital d'instruction des Armées Begin, 94160 Saint Mandé, France*

Received 13 February 1981

1. Introduction

During a survey for hepatitis, an abnormal hemoglobin was found in a 26 year old man. This hemoglobin (38% of the total) migrated, on cellulose acetate strips and on isoelectric focusing (IEF), like Hb F. Structural and functional studies showed that this abnormal hemoglobin was a new low oxygen affinity mutant: Hb Saint Mandé $\alpha_2\beta_2$ 102 (G4) Asn → Tyr. This mutation is localized in the $\alpha_1\beta_2$ contact.

2. Material and methods

Hematological data were obtained using standard methods. Blood for structural studies was collected in ACD.

The abnormal fraction was detected by electrophoresis on cellulose acetate strips at pH 8.6 and by IEF as in [1]. Hemoglobin A_2 quantification, citrate agar electrophoresis, the isopropanol stability test, and electrophoresis of globin chain in 6 M urea buffer at pH 6 and 9 were performed using standard techniques.

For structural studies the abnormal fraction was purified by preparative IEF. Techniques for globin precipitation, chain separation, aminoethylation, tryptic hydrolysis, analytical and preparative fingerprints and amino acid analysis have been described [2].

Abnormal βT-11 was isolated by column chromatography on Aminex A-5 and purified on a column of Bio-Rad AG 50 X 4. Manual sequential analysis of amino acid residues was performed by Edman degradation and dansylation as in [3]. Carboxypeptidases (A+B) from Worthington were used as in [4].

Oxygen dissociation curves in whole blood were measured at 37°C at the pH of the blood with an Hem-O-Scan analyser (American Instrument Co.).

The 2,3-diphosphoglycerate concentration was determined by the enzymatic method in [5].

3. Results

3.1. *Case report and hematological studies*

A French caucasian 26-year-old male was referred to hospital for a hepatitis examination. A slight anemia was found (table 1). The clinical examination showed only a labial cyanosis. In order to explain this cyanotic anemia, some biological analyses were performed: serum iron, cobalamins, folates and haptoglobin, were

Table 1
Hematological data of the propositus

	RBC ($\times 10^{12}$/l)	Hb (g/dl)	PCV (l/l)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Reticulocytes ($\times 10^9$/l)	Ferritin (μg/l)	Hb Saint Mandé (%)	Hb A2 (%)
a	3.16	9.6	31.4	99	30.4	30.8	2	–	38.5	3
b	3.92	11.8	35.9	91	30.1	32.8	1	44	–	–

[a]During hepatitis A; [b]after complete improvement

all normal. Bone marrow smears were also normal. Blood gas analysis was performed: PaO_2, 106 mm Hg; measured SaO_2, 81%.

Hemoglobin analysis by cellulose acetate strips showed an abnormal fraction (38%) migrating in the position of Hb F. By Betke's method, Hb F concentration was 0.5%. The isopropanol stability test was normal.

Some other members of the propositus's family were studied. The mother displayed the same hematological and electrophoretical features.

3.2. *Structural studies*

Routine isoelectric focusing of the hemolysate over pH 6–9 revealed an abnormal band, migrating on the cathode side of the Hb A band, close to Hb F. This abnormal hemoglobin showed a pI higher than those of two control variants, Hb Hotel-Dieu [6] and Hb Pitie-Salpetriere [7] (fig.1). Electrophoresis of the globin in 6 M urea at pH 8.9 and pH 6.0 did not reveal any difference from the globin A control.

The abnormal hemoglobin was separated by preparative IEF. Ampholytes were removed from the purified hemoglobin by column chromatography on a mixed bed resin of AG 501 X 8 (Bio-Rad). According to the percentage of the abnormal component and in the absence of doubling of the Hb A_2 band, study of the β-chain was performed. The fingerprint of the tryptic hydrolysate of the aminoethylated β-chain was normal, except for the absence of the βT-11 peptide. The βT-13 was quantitatively increased in Hb Saint Mandé as compared to the βT-13 of the β^A chain control (fig.2). This spot which stained for Tyr, also stained positively for His and Arg, suggesting that this peptide was a mixture of the normal βT-13 and the abnormal βT-11.

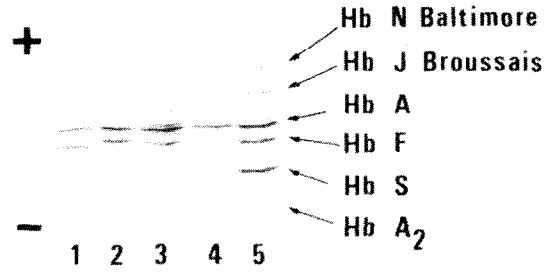


Fig.1. Isoelectric focusing in a pH gradient range 6 → 9: (1) hemolysate Saint Mandé; (2) hemolysate Hôtel Dieu; (3) hemolysate Bougardirey Mali; (4) hemolysate Pitié Salpêtrière; (5) control mixture

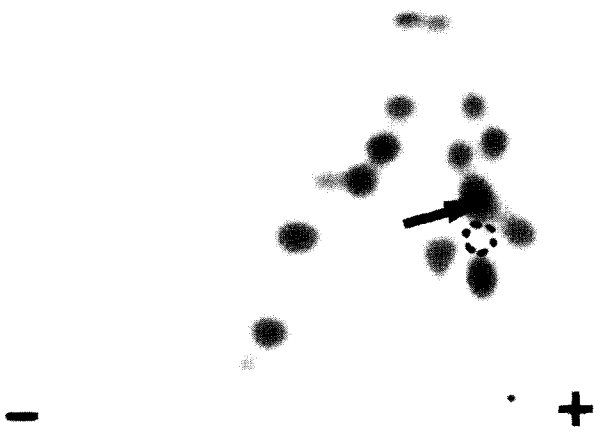


Fig.2. Fingerprint at pH 6.4 of a tryptic digest of β-aminoethylated chain from Hb Saint Mandé. The dotted circle indicates the position of the normal βT-11; the arrow shows the position of the new peptide according to the positive staining for arginine.

To separate the βT-11 peptide, preparative chromatography on Aminex A5 was performed. The elution pattern obtained was normal excepted for the zone of the βT-11 which eluted more slowly than the βT-11 of the control (fig.3). Amino acid analysis revealed the presence of a peptide with the amino acid composition of the βT-11, except for the presence of tyrosine and the lack of one aspartyl residue.

On account of the presence of one aspartyl and one asparagine residue in the normal peptide, a manual Edman-dansyl degradation was performed. The Asp residue β99 was present but it was uncertain as to whether the Tyr residue β102 was present. A digestion of the peptide with a mixture of carboxypeptidase A and B was thus performed. The results obtained gave the following sequence:

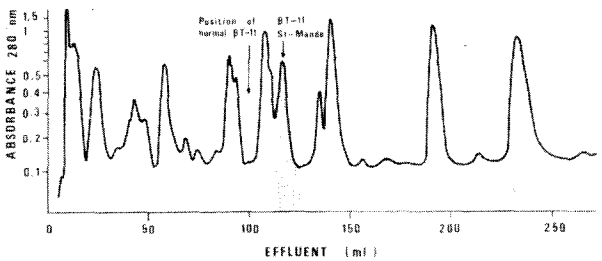
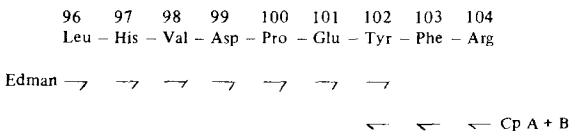


Fig.3. Elution pattern of the tryptic hydrolysate of β-aminoethylated Saint Mandé chain on a column of Aminex A5.



Hb Saint Mandé therefore contains the amino acid substitution β102 Asn → Tyr. This substitution is in accordance with the mRNA sequence of the gene for the normal β-chain [8].

3.3. *Functional studies*

The oxygen affinity curve of the red blood cells was markedly shifted to the right; the P50 was 52 mm Hg (normal value: 28 ± 1 mm Hg). The red cell concentration of 2,3-diphosphoglycerate was 16.95 μM/g Hb as compared to 15 ± 2 μM/g Hb in the control.

These results indicated that the red blood cells contained a low oxygen affinity hemoglobin. Detailed functional studies will be published later.

4. Discussion

Only a few abnormal hemoglobins have been reported in which a reduced oxygen affinity occurs. Three hemoglobin variants have already been described for residue β102: Hb Richmond (Asn → Lys) [9]; Hb Kansas (Asn → Thr) [10]; and Hb Beth Israel (Asn → Ser) [11,12]. Hb Kansas and Hb Beth Israel present a low oxygen affinity as does Hb Saint-Mandé. Hb Richmond has been shown to have increased tetramer–dimer dissociation and its oxygen affinity has been inferred to be normal.

The normal residue β102 Asn (G4) forms an hydrogen bond with α94 Asp (G1). Any interference with this bond shifts the R ⇌ T equilibrium towards the T state, resulting in a lowered oxygen affinity.

Hemoglobins Beth Israel and Kansas are associated with a cyanosis as in the case of Hb Saint-Mandé. The apparent anemia found in this family requires further investigation.

Acknowledgements

We thank Dr J. Chapman for assistance in reviewing the manuscript and A. M. Dulac and M. Segear for preparing the manuscript. This work was supported in part by l'Institut National de la Santé et de la Recherche Médicale, CRL no. 79 5 131 and by la Delegation Générale à la Recherche Scientifique et Technique no. de la cystamine 78 7 0345.

References

[1] Beuzard, Y., Basset, P., Braconnier, F., El Gammal, H., Martin, L., Oudart, J. L. and Thillet, J. (1975) Biochim. Biophys, Acta 393, 182–187.
[2] Blouquit, Y., Arous, N., Machado, P. E. A., Perrone, F. and Garel, M. C. (1976) FEBS Lett. 72, 5–7.
[3] Weiner, A. M., Platt, T. and Weber, K. J. (1972) J. Biol. Chem. 247, 3242–3251.
[4] Narita, K., Matsuo, H. and Nakajima, T. (1975) Protein Sequence Determination, Springer-Verlag, Berlin, New York.
[5] Rose, Z. B. and Leibowitz, J. (1970) Anal. Biochem. 35, 177–180.
[6] Blouquit, Y., Braconnier, F., Galacteros, F., Arous, N., Soria, J., Zittoun, R. and Rosa, J. submitted.
[7] Blouquit, Y., Braconnier, F., Cohen-Solal, M., Foldi, J., Arous, N., Ankri, A., Binet, J. L. and Rosa, J. (1980) Biochim. Biophys. Acta 624, 473–478.
[8] Marotta, C. A., Forget, B. G., Cohen-Solal, M., Wilson, J. T. and Weissman, S. H. (1977) J. Biol. Chem. 252, 5019–5031.
[9] Efremov, G. D., Huisman, T. H. J., Smith, L. L., Wilson, J. B., Kitchens, J. L., Wrishstone, R. N. and Adams, H. R. (1969) J. Biol. Chem. 244, 6105–6116.
[10] Bonaventura, J. and Riggs, A. (1968) J. Biol. Chem. 243, 980–991.
[11] Nagel, R. L., Lynfield, J., Johnson, J., Landau, L., Bookchin, R. and Harris, M. (1976) New Engl. J. Med. 295, 125–130.
[12] Efremov, G. D., Stojmirovic, E., Lam, H. L., Wilson, J. B. and Huisman, T. H. J. (1978) Hemoglobin 2, 75–77.