STRUCTURAL AND FUNCTIONAL STUDIES OF Hb ROTHSCHILD β 37 (C3) TRP → ARG

A new variant of the $\alpha_1\beta_2$ contact

Gérard GACON, Olga BELKHODJA, Henri WAJCMAN and Dominique LABIE
Institut de Pathologie Moléculaire INSERM U 15, CHU Cochin, 24, Rue du faubourg Saint-Jacques, 75014 Paris

and

Albert NAJMAN
Service d'Hématologie, CHU Saint-Antoine, 75012 Paris, France

Received 19 August 1977

1. Introduction

The $\alpha_1\beta_2$ contact area of the hemoglobin molecule is known to be involved in the structural transition that occurs on oxygenation [1,2] and is of critical importance for the full expression of its allosteric properties [3]. This has been confirmed by the functional disorders observed in several mutants with substitution in the $\alpha_1\beta_2$ region [4] including Hb Hirose β 37 (C3) Trp → Ser, the unique example of a substitution of the β 37 tryptophan residue [5].

In this paper, we describe the structural study of a new mutant, Hb Rothschild*, in which the same tryptophan residue is replaced by an arginine. Its oxygen equilibrium properties are also reported.

2. Material and methods

2.1. *Structural studies*

Blood was collected on heparin and hemolysates were prepared by routine procedures. Electrophoreses were performed on cellulose acetate plates (Helena Laboratories) in Tris–EDTA buffer, pH 8.8.

Isoelectrofocusing on polyacrylamide gels was done as described by Drysdale et al. [6]. The abnormal component was purified on DEAE–Sephadex in 0.05 M Tris–HCl buffer, using a pH gradient from 8.2–7.5.

After removal of the heme, the globin chains were separated according to Clegg et al. [7].

The tryptic digest was fingerprinted on cellulose thin-layer plates. For preparative purposes, silica-gel plates of 0.5 mm thickness were used, allowing direct elution of the abnormal peptides. The amino acid composition of peptides was determined after hydrolysis by HCl, using a Beckman 120 C amino acid analyzer.

2.2. *Functional studies*

Oxygen equilibrium curves were determined by the discontinuous spectrophotometric technique of Benesch et al. [8]. The pure components were stripped on a Dintzis column [9] and studied in 0.05 M bis–Tris buffer.

3. Results and discussion

Electrophoresis of the hemolysate at alkaline pH showed an abnormal fraction migrating between HbS and HbA_2. A similar pattern was obtained by isoelectrofocusing (fig.1). The abnormal fraction eluted from DEAE–Sephadex at pH 8.0 as compared to HbA at pH 7.7, and constituted 47% of the total hemoglobin.

*Name given from the Hôpital Rothschild, Paris

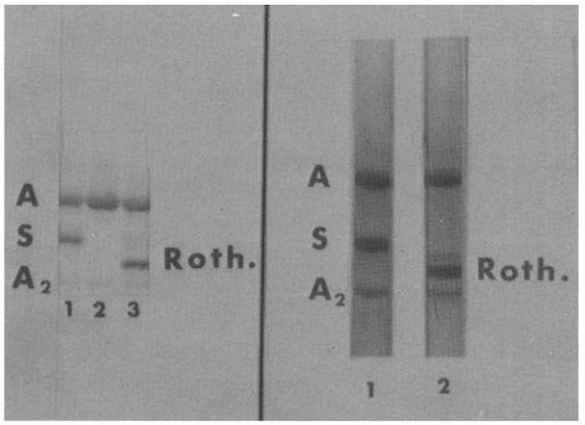

Fig.1. Comparative patterns of lysates containing Hb Rothschild and HbS obtained by cellulose acetate electrophoresis (left) and gel isoelectrofocusing (right). Left: 1, HbA/S; 2, normal lysate; 3, HbA/Rothschild. Right: 1, HbA/S; 2, HbA/Rothschild.

On chain separation, one abnormal β chain with increased positive charge was observed; the elution pattern showed a decreased absorbance ratio at 280 nm between β and α chains, suggesting the absence of an aromatic residue in the abnormal β chain.

No abnormality could be detected on the ninhydrin-stained peptide map (fig.2). But specific staining for arginine revealed the presence of a supplementary spot, not shown by ninhydrin. Also, on staining with Ehrlich's reagent, no tryptophan was found in the βT_4 spot. After elution from a preparative fingerprint, both abnormal peptides were analyzed (table 1). The

Table 1
Amino acid composition of the two abnormal peptides eluted from a preparative fingerprint

Amino acid	Hb Rothschild	HbA	HbA
	βT_4A	βT_4B	βT_4
Thr		0.9	1
Glu		1	1
Pro	0.9		1
Val	2.0		2
Leu	1.9		2
Tyr	1		1
Arg	1	1	1
Trp	0	0	1

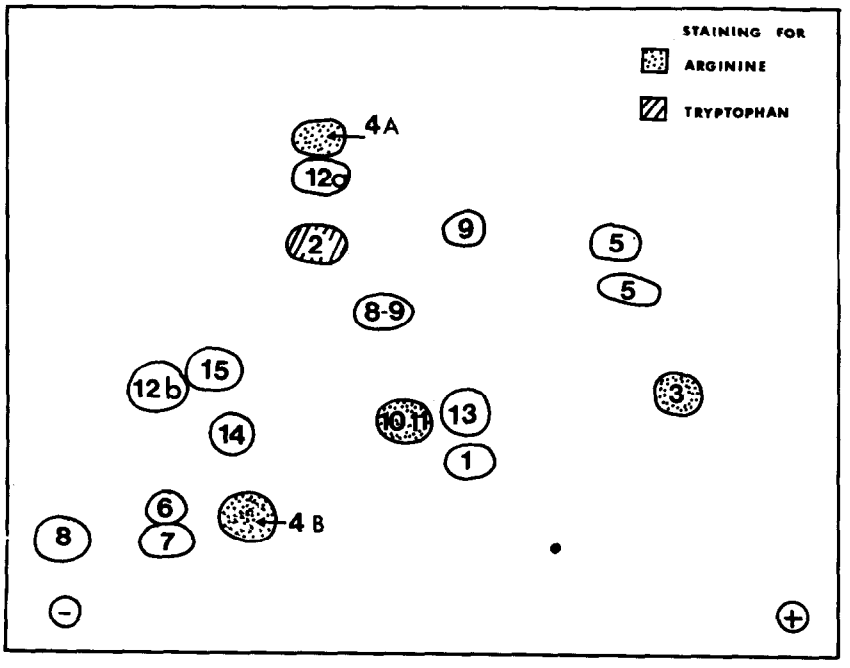

Fig.2. Fingerprint of the β chain on cellulose thin-layer. A supplementary spot, βT_4B, stained for arginine is observed; the peptide βT_4A stained for arginine but not for tryptophan.

Table 2
Amino acid sequence of βT_4A and βT_4B peptides of Hb Rothschild as compared to the normal βT_4

βA	Leu–Leu–Val–Val–Tyr–Pro–Trp–Thr–Gln–Arg	
	31	37
β Rothschild	Leu–Leu–Val–Val–Tyr–Pro–Arg–Thr–Gln–Arg	
	βT_4A	βT_4B

results indicate that peptide βT_4 has been specifically cleaved by trypsin, due to the replacement of tryptophan by arginine, as illustrated in table 2. This is therefore a new variant, Hb Rothschild, β37 tryptophan → arginine.

The oxygen equilibrium curve obtained on pure stripped Hb Rothschild demonstrated a decreased oxygen affinity, the P_{50} being equal to 3.5 mm Hg instead of 2 mm Hg for control HbA. The heme–heme interaction was also found reduced ($n - 1.8$).

The β37 tryptophan residue lies in the $\alpha_1\beta_2$ contact area, in both oxy and deoxy structure [1,2]. Moreover, Perutz et al. have given spectral evidence of its involvement in the T and R structural transition [10].

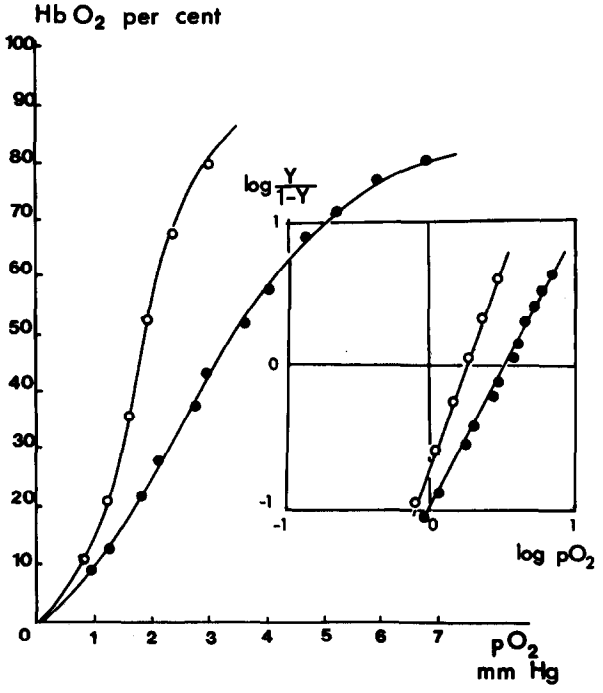

Fig.3. Oxygen equilibrium curves of pure stripped hemoglobins. Bis–Tris 0.05 M buffer, pH 7.1, 25°C. Hb Rothschild (—•—), HbA (—○—).

Hence, taking into account the size and the strongly apolar character of the tryptophan residue, any substitution should lead to structural perturbations of the $\alpha_1\beta_2$ contact.

From the initial functional studies it is apparent that the allosteric transition is disturbed by the substitution.

In Hb Hirose a similar impairment of the cooperativity was observed [5], but the oxygen affinity was increased as opposed to the decrease observed in Hb Rothschild. This discrepancy can probably be explained by the difference in size and charge between the serine residue present in Hb Hirose and that of arginine in Hb Rothschild. The positively charged, guanidininium group of the newly introduced arginine could be involved in electrostatic interactions that would stabilize the T structure.

Acknowledgements

We thank Dr R. W. Carrell for critical reading of the manuscript and helpful discussion. This work was supported by a grant from the Institut National de la Santé et de la Recherche Médicale (No. 76 507 43).

References

[1] Perutz, M. F., Muirhead, H., Cox, J. M. and Goaman, L. C. G. (1968) Nature 219, 131–139.
[2] Fermi, G. (1975) J. Mol. Biol. 97, 237–256.
[3] Perutz, M. F. (1970) Nature 228, 726–734.

[4] Bellingham, A. J. (1976) Brit. Med. Bull. 32, 234–238.
[5] Yamaoka, K. (1971) Blood 38, 730–738.
[6] Drysdale, J. W., Righetti, P. G. and Bunn, H. F. (1971) Biochim. Biophys. Acta 229, 42–50.
[7] Clegg, J. B., Naughton, M. A. and Weatherall, D. J. (1966) J. Mol. Biol. 19, 91–108.
[8] Benesch, R., MacDuff, G. and Benesch, R. E. (1965) Anal. Biochem. 11, 81–87.
[9] Nozaki, C. and Tanford, C. (1967) Meth. Enzymol. 11, 715–734.
[10] Perutz, M. F., Ladner, J. E., Simon, S. R. and Ho, C. (1974) Biochemistry 13, 2163–2174.