STRUCTURAL AND FUNCTIONAL STUDIES OF HAEMOGLOBIN SURESNES OR α_2 141 (HC3) Arg → His β_2, A NEW HIGH OXYGEN AFFINITY MUTANT

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1. Introduction

Structural and functional studies of mutant haemoglobins have been of importance to the understanding of the molecular mechanisms involved in the functioning of normal human haemoglobin [1]. In this regard the functional importance of the C-termini (His^{146} HC3) of the β-chains has been demonstrated in three abnormal haemoglobins, namely: Hb Hiroshima (α_2 β_2 146 (HC3) His → Asp) [2], Hb Cochin-Port Royal (α_2 β_2 146 (HC3) His → Arg) [3] and Hb York (α_2 β_2 146 (HC3) His → Pro) [4]. The alterations in the function of these abnormal haemoglobins seem to support the steric model proposed by Perutz for HbA [5]. In this model the C-termini of the β- and α-chains are responsible for salt bridges with neighbouring amino acids involved in the stabilization of the T or deoxy structure and the stereochemistry of the Bohr effect [5]. Only one abnormal haemoglobin with a substitution on the C-termini of the α-chains has been described, Hb Singapore (α_2 141 (HC3) Arg → Pro β_2) [6] but to our knowledge without any functional studies.

This report concerns studies on the structure and functioning of a new mutant (α_2 141 (HC3) Arg → His β_2) which appears of great interest as the role of the Arginine α 141 is not completely understood. It has been denominated Hb Suresnes and is characterized by a 6-fold increase in O_2 affinity, a reduced Bohr effect and low cooperativity which is partially restored by organic phosphates. Similar results were obtained in O_2 binding studies of whole red cells.

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2. Material and methods

Haematological data were obtained by routine Coulter counter analysis.

2.1. *Structural studies*

Electrophoresis was performed on cellulose acetate plates (Helena Laboratories) in Tris–EDTA buffer, pH 8.8. Analytical globin electrophoresis was performed at alkaline pH in 6 M urea barbital–Tris–EDTA buffer according to Cortesi et al. [7]. Isoelectrofocusing was done as described by Drysdale et al. in 3 mm I.D. tubes [8]. The abnormal component was purified on a DEAE–Sephadex column using 0.05 M Tris–HCl buffer with a pH gradient from 8 to 7.40. Deheminisation was achieved on the whole lysate and the polypeptide chains were then separated according to Clegg et al. [9]. The tryptic digest was finger-printed on silica gel thin layers and stained by ninhydrin and various specific stains. The same tryptic digest was chromatographed on cation exchange resin (Beckman IA. 35) according to Jones [10]. Further purification was done on Aminex Ag 50 WX_2 and the amino acid composition determined on a Beckman 120 C amino acid analyser.

2.2. *Functional studies*

The oxygen affinity of washed fresh red cells was measured by the continuous recording of the oxygen dissociation curve in an isotonic phosphate bicarbonate

Table 1
Haematological data on the members of the family avalaible for investigation

Generation	Sex	Age (Y)	Hb g/dl	RBC × 10^{12}/l	PCV	MCV (fl)	MCH (pg)	MCHC (g/dl)
I.1	m	50	15.3	4.7	0.427	91	32.0	35.3
I.2[a]	f	45	15.1	5.0	0.438	89	30.1	34.4
II_1[a]	m	15	16.5	5.4	0.449	83	29.8	36.3
II_2[a]	m	6	15.9	5.5	0.439	81	28.5	36.0

[a]Carriers of the Hb Suresnes.

buffer at 37°C using the DCA Radiometer according to Teisseire et al. [11]. The abnormal haemoglobin was stripped on a Dintzis column [12]. Functional studies of purified Hb Suresnes were performed with the discontinuous spectrophotometric technique of Benesch et al. [13] in 0.05 M Tris or bis-Tris buffer in 0.1 M NaCl at 30°C. Intra-erythrocytic 2,3-diphosphoglycerate (DPG) was measured by the technique of Rose and Leibowitz [14]. Additions of DPG or inositolhexaphosphate (InP_6) were as the sodium salts.

3. Results and discussion

3.1. *Clinical and haematological data*

Hb Suresnes was found by electrophoresis of the hemolysate of the blood of a 6-year-old boy who was undergoing surgery in our hospital and who was found by routine haematological examination to have an abnormal RBC count and Hb concentration. Among the members of the family who have been studied so far, the mother and the second elder son have the same electrophoretic pattern as the propositus (table 1) No clinical symptoms can be related in these three healthy individuals to the abnormal haemoglobin.

3.2. *Structural characterization*

By routine electrophoresis an abnormal component was detected moving more anodically than HbA, like HbJ. Another minor component was seen more anodic than HbA_2 indicating a mutation in the α-chains. This was further confirmed by the electrophoretic pattern of the globin chains in 6 M urea buffer at alkaline pH which showed two separated α-chains. Paradoxically the isoelectric point of the abnormal component was only slightly more acid than that of HbA (HbA: 6.95, Hb Suresnes: 6.91, HbJ Mexico: 6.74) (fig.1).

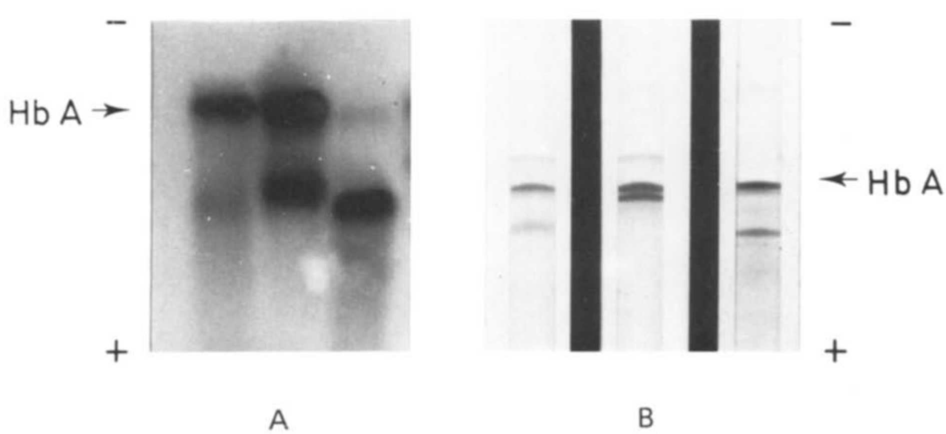

Fig.1. Comparative patterns of Hb Suresnes and other fast mutants by routine electrophoresis and by isoelectrofocusing. A: electrophoresis on cellulose acetate plates. From left to right: Hb J Mexico, Hb Suresnes, HbJ Baltimore. B: isoelectrofocusing in polyacrylamide gels (pH 6.8). From left to right: HbJ Hofu, Hb Suresnes, HbJ Mexico. The striking difference in migration between these two procedures could be indicative of the involvement of a His in the substitution.

The abnormal component eluted from the DEAE–Sephadex just after HbA and amounted to 39% of the total. Thinking that the difference in charge would be sufficient to allow separation of normal and abnormal α-chains, chain separation was performed on the unfractionated globin. Surprisingly there was only one β- and one α-peak both in their normal positions. Comparison of these various electrophoretic and chromatographic patterns at various pHs suggested a substitution of histidine for arginine which was the only possibility according to the genetic code. Since there are only three arginine residues in normal α-chains, the mixture obtained could be used for structure determination. The total α-chain fraction was digested by trypsin and analysed. On the fingerprint of this mixture a supplementary spot was seen that stained for both tyrosine and histidine, roughly at the same position as the normal β-T_{15} (Tyr–His) peptide. The difference was more noticeable on the cation exchange pattern. All the peaks of normal α chains were present and a supplementary peak was noticed between α-T_3 and α-T_{10} eluting like β-T_{15} (fig.2). After further purification this peak was identified as a dipeptide Tyr–His. The abnormality was therefore α 141 (HC3) Arg → His. It is noteworthy that the method of identification of this variant was exactly the reverse of that previously used for Hb Cochin-Port Royal β 146 (HC3) His → Arg (fig.2) [3].

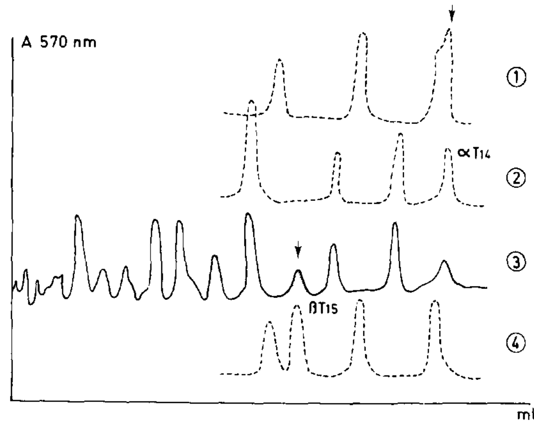

Fig.2. The cation exchange tryptic peptides elution patterns of (from top to bottom) 1: βAE Cochin-Port Royal. 2: α-normal. 3: α-normal + α-Suresnes. 4: βAE normal. The abnormal peptide of α-Suresnes (hatched) is eluted at the same volume as that of normal β-T_{15} (Tyr–His). The reverse observation was made for Hb Cochin-Port Royal when compared to a normal α-chain [3].

3.3. *Functional studies*

The oxygen affinity of intact fresh red cells is increased: P_{50} at pH 7.40, PCO_2: 5.32 kPa (40 mmHg) = 2.33 kPa (17.5 mmHg) compared to our normal value for adult blood: 3.66 kPa (27.5 ± 1 mmHg). The cooperativity estimated from the slope of the Hill plot is decreased, n_H at P_{50} = 2.06 (control = 2.78). Red

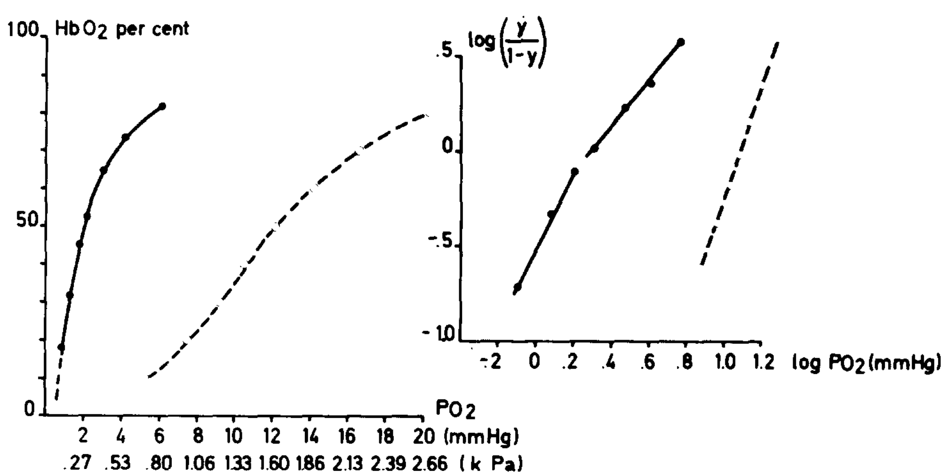

Fig.3. Oxygen affinity of the stripped pure haemoglobin isolated by DEAE–Sephadex. Bis–Tris 0.05 M buffer in 0.1 M NaCl, pH 7.13, 30°C. Hb Suresnes (–•–); Hb A (– –o– –). From the Hill plot, the slope of Hb Suresnes is biphasic. (1 mmHg = 0.133 kPa).

Table 2
The influence of pH, DPG and InP_6 on the oxygen affinity and cooperativity of Hb Suresnes

	log P_{50} of 'stripped' Hb	
	Hb Suresnes	Hb A
pH 6.84	0.391	1.229
7.13	0.273	1.092
7.33	0.225	1.005
7.64	0.179	0.874
$\Delta \log P_{50}/\Delta pH$	−0.262	−0.443
n_H at P_{50}	1.26	2.9
DPG added (pH 7.13)	0.427	1.307
Molar ratio DPG/Hb_4 = 12	(+ 0.154)	(+ 0.215)
n_H at P_{50}	1.44	2.9
InP_6 added (pH 7.13)	0.832	1.735
Molar ratio InP_6/Hb_4 = 4	(+ 0.559)	(+ 0.643)
n_H at P_{50}	2.16	2.2

cell [DPG] is normal: 0.78 mmol/mmol Hb_4 (control = 0.85 ± 0.10).

The oxygen affinity of pure 'stripped' Hb Suresnes is increased approximately 6-fold P_{50} = 0.253 kPa (1.9 mm Hg) compared to HbA for which P_{50} = 1.64 kPa (12.3 mm Hg) when studied in the same conditions (pH 7.13, 30°C) (fig.3). The Hill plot for Hb Suresnes shows a low cooperativity (n_H = 1.26 at P_{50}) and a biphasic slope which cannot be explained from the present results. Similar observations were made at all pH values studied, from 6.84 to 7.64. The Bohr effect ($\Delta \log P_{50}/\Delta$ pH) is decreased by 30–40% approximately (table 2). After the addition of DPG or InP_6 an increase of P_{50} of Hb Suresnes is observed but this effect appears to be somewhat lower than that measured in solutions of pure HbA. Cooperativity of Hb Suresnes is increased by DPG and almost restored to normal by InP_6 (table 2).

As expected from the location of the amino acid substitution at the C termini of the α-chains, these results indicate that the salt bridges which normally form on deoxygenation are either less stable or disrupted in deoxy Hb Suresnes. When compared to artificially modified [15] or naturally occurring C termini mutants, the present report emphasizes the importance of the specific chemical nature of the substitution.

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