THE OXYGEN AFFINITY OF HAEMOGLOBIN ST. LUKE'S

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1. Introduction

Haemoglobin (Hb) St. Luke's (α95 (G2) Pro → Arg) was found by Bannister et al. [1, 2] in three Maltese families. Position α95 forms part of the tetrameric α1 β2 contact [3] which is involved in the conformational changes associated with oxygenation and deoxygenation of the Hb molecule [4]. Three other variants with substitutions in that position, Hb G Georgia (leu) [5], Rampa (Ser) [6], and Denmark Hill (Ala) [7], have a raised oxygen affinity in phosphate buffer [7, 8].

We have recently found a case of Haemoglobin St. Luke's, again in a Maltese man who had polycythaemia. As polycythaemia is sometimes associated with a high-affinity haemoglobin [9] we have examined the oxygen affinity of isolated Hb St. Luke's in phosphate buffer and have found it to be increased.

2. Materials and methods

2.1. *Isolation of the haemoglobin*

Electrophoresis was carried out on paper using Tris buffer, pH 8.9 [10] and discontinuous Tris–barbiturate buffer, pH 8.5 [11], and on starch gel at pH 8.6 [12]. The variant Hb was isolated in the carbon monoxy (CO) form by chromatography on Sephadex CM50 [13] and was further purified on DEAE-Sephadex [14]. The isolated haemoglobin contained 11.7% methaemoglobin (metHb), which was reduced with dithionite by the method of Dixon and McIntosh [15] in CO-saturated 0.05 M K_2HPO_4, pH 8.6.

2.2. *Measurement of the oxygen affinity*

The purified CO–Hb solution from both the St. Luke's and the A fractions were adjusted to a concentration of 2–3% and converted to oxyhaemoglobin by illumination in the presence of oxygen [16]. The oxygen dissociation curves were determined by the method of Imai et al. [17] using 0.1% Hb solution in 0.1 M potassium phosphate buffers containing 5 mM EDTA at 20°C.

2.3. *Identification of the substitution*

The purified Hb was converted to globin and separated into α and β chains [18, 7]. Fingerprints of tryptic digests of the unmodified α-chain were prepared [19] and stained with ninhydrin and reagents for specific amino acids [20]. Relevant peptides were eluted from preparative fingerprints [21] and hydrolysed with contant-boiling HCl in sealed tubes at 108°C for 18 hr; where necessary, peptides were further purified by paper electrophoresis at pH 3.5 before elution. The dried hydrolysates were analysed using a Locarte amino acid analyser.

3. Results

The blood of the carrier indicated a mild polycythaemia: Hb, 16.4 g/dl; RBC 5.98 × $10^6/\mu l$; PCV 48%; MCV 80 fl; MCH 27 pg; reticulocytes less than 1%.

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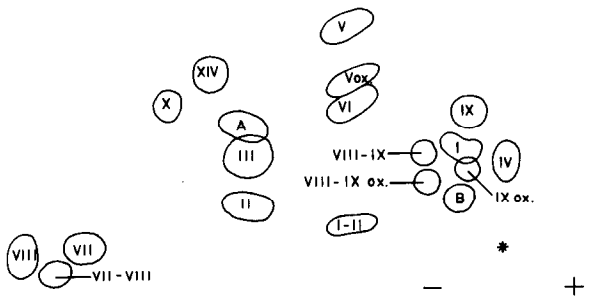

Fig. 1. Fingerprint of the soluble tryptic peptides from the α-chain of Hb St. Luke's. Electrophoresis at pH 6.4, 53 V/cm for 50 min; ascending chromatography in pyridine–isoamyl alcohol–water (6:6:7, by volume) for 18 hr.

Residue No.	93	94	95	96	97	98	99
Helical No. Residues:	FG5	G1	G2	G3	G4	G5	G6
Hb A	Val	Asp	Pro	Val	Asn	Phe	Lys
Hb St. Luke's	Val	Asp	ARG	Val	Asn	Phe	Lys

Fig. 2. Amino acid sequence of the tryptic peptide XIIb of the β-chain of Hb A and Hb St. Luke's

Hb St. Luke's separated from Hb A on starch gel electrophoresis and during column chromatography on Sephadex CM50, from which the amount was estimated to be 14% of the total haemoglobin; it hardly separated from Hb A on paper electrophoresis at pH 8.9 or in a discontinuous Tris system, pH 8.5, and did not separate on DEAE-Sephadex chromatography. All methods revealed a second Hb A_2, which migrated just behind the normal Hb A_2 on starch gel and separated particularly well on discontinuous paper electrophoresis; this suggested that Hb St. Luke's differed from Hb A in its α chains.

Fig. 1 shows the fingerprint of the soluble tryptic peptides of the isolated α chain, which differed from that of the normal α chain by two new spots (A and B in fig. 1). Peptide A was positively-charged, gave no specific staining reaction and partly overlapped αTpIII, from which it could be purified by paper electrophoresis at pH 3.5; peptide B was neutral and gave a positive staining reaction for arginine. The amino acid composition of the two peptides, shown in table 1, can be accounted for by a Pro → Arg substitution at position α95 in TpXI (α93–99). This introduces a

Table 1
Amino acid analyses of tryptic peptides A and B from Hb St. Luke's.

Amino acid	A nmoles	A Molar ratios	B nmoles	B Molar ratios
Aspartic acid	45.66	0.96	84.36	1.01
Valine	49.71	1.05	82.62	0.99
Phenylalanine	47.92	1.01		
Lysine	46.13	0.97		
Arginine			82.61	0.99

Table 2
Oxygen affinity data for Hb A and Hb St. Luke's.

				% metHb*
Hb A	pH	p_{50}	$\log_{10} p_{50}$	Before / After
	5.98	13.0	1.114	16.8 / 25.6
	6.47	14.6	1.164	5.0 / 12.6
	6.96	10.4	1.105	5.8 / 10.6
	7.37	6.2	0.789	6.3 / 9.0
	7.83	2.7	0.431	5.6 / 8.3
Hb St. Luke's				
	6.02	5.3	0.720	12.0 / 33.8
	6.48	5.2	0.716	10.0 / 16.5
	6.97	4.3	0.634	Not determined
	7.38	2.8	0.447	10.7 / 13.0
	7.85	1.6	0.204	10.5 / 13.0

*Determined spectrophotometrically before and after each measurement.

new site susceptible to tryptic hydrolysis, resulting in two new peptides: α93–95, which would be an electrically neutral arginine peptide and α96–99, which would be positively charged at pH 6.4 and would give no specific staining reactions (see fig. 2). (The absence of α^{A}TpXI is not apparent from the fingerprint because it normally overlaps αTpI and gives no specific staining reactions). Thus the variant is Hb St. Luke's, α95 (G2) Pro → Arg.

Table 2 records the values of p_{50}, $\log_{10} p_{50}$ and the metHb concentration for both the normal (Hb A) and St. Luke's fractions. The values of n, the Hill coefficient, were in the range 2.5–2.6 for the A fraction, and 1.9–2.0 for the St. Luke's fraction; the latter is

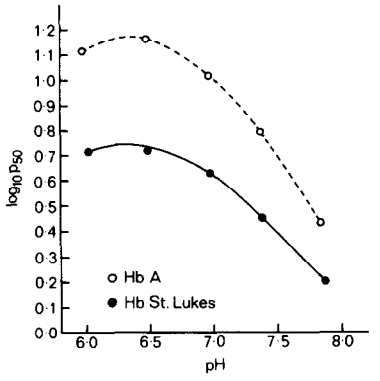

Fig. 3. The variation of $\log_{10}p_{50}$ with pH for Hb St. Luke's and Hb A. p_{50} is the oxygen pressure, in mm Hg, at which the Hb is 50% saturated with O_2.

probably less than the true value because of the relatively large metHb content of the sample.

Fig. 3 shows the variation of $\log_{10}p_{50}$ with pH for St. Luke's and Hb A. The slope of these curves (d $\log_{10}p_{50}$/d pH) gives a measure of the Bohr effect; it can be seen that the curves are approximately parallel, but the slope of the curve between pH 7.0 and pH 7.9 was rather less for Hb St. Luke's than for Hb A. The Bohr factor $\Delta \log_{10}p_{50}/\Delta$ pH between pH 7.0 and pH 7.4 is −0.54 for Hb A and −0.46 for Hb St. Luke's. Thus, the oxygen affinity of Hb St. Luke's is increased, being about 2.5 times that of Hb A at pH 7.4; the haem–haem interaction is reduced and the Bohr effect is slightly reduced.

4. Discussion

Bannister et al. [1] reported that Hb St. Luke's appeared to have a slightly decreased oxygen affinity, but considered the value of their data limited by the high concentration (26%) of metHb in their samples. Our measurements with a sample containing 10–13% metHb showed that Hb St. Luke's has a raised oxygen affinity.

In addition to having a raised oxygen affinity, Haemoglobins G Georgia (α95 Leu), Rampa (α95 Ser) and St. Luke's (α95 Arg) all show an increased tendency for the liganded form of the tetramer to dissociate into dimers [1, 8], which presumably results from the weakening of the α1 β2 contact. The four variants with substitutions at α95 show anomalous electrophoretic behaviour; Haemoglobin G Georgia, Rampa and Denmark Hill, in which proline α95 is replaced by a neutral residue, all migrate more slowly than Hb A during starch gel or cellulose acetate electrophoresis at pH 8.6. Surprisingly, Hb St. Luke's, in which proline is replaced by arginine, separates no better from Hb A on paper electrophoresis at pH 8.9, despite the introduction of two additional positive charges per tetramer. Dr. M.F. Perutz has suggested a possible explanation for this. The α95 side chains protrude into the internal cavity towards a position on the two-fold symmetry axis where the electron density map of oxy, but not deoxy, Hb A shows a small peak, which he attributes to a weakly bound anion (probably SO_4^{2-} from the 2 M $(NH_4)_2SO_4$ solution from which the crystals were precipitated). The presence of the two extra arginines might lead to strong binding of anions, such as phosphate, in this position. This would neutralise the positive charges of the arginine side chains and thus account for the poor electrophoretic separation.

The high oxygen affinity of Hb St. Luke's could result from the disturbance of the α1 β2 interface brought about by the absence of the pyrollidone ring of Pro α95 (which makes contacts with Trp β37 [6]). Preferential binding of phosphate to the oxy form might also contribute to the raised oxygen affinity.

All the carriers of Hb St. Luke's described by Bannister et al. [1, 2] were reported to be clinically and haematologically normal; our subject, however, was diagnosed as having polycythaemia and had to have a toe amputated because of vascular occlusion. He was a heavy smoker, and had diabetes, but polycythaemia, which is known to cause vascular occlusion on its own account [22], may well have been a contributory factor.

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