Haemoglobin Warwickshire (β5 [A2] Pro → Arg)

A possible 'fine tuning' of 2,3-DPG affinity by β5 Pro

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The blood of a 67-year-old Scotsman who was admitted to hospital with an abdominal carcinoma showed an abnormal red cell lysis in the oxidase channel of the Technicon H6000 analyser. This is seen regularly with Hb C and sometimes with Hb S and on further investigation a new haemoglobin variant – Hb Warwickshire – was found. When the family was examined, other examples were revealed. The haematological parameters were normal. Hb Warwickshire is mildly unstable and whilst in the absence of phosphates its oxygen dissociation does not differ from that of Hb A, a small fall of oxygen affinity is noted when phosphates are present.

Human hemoglobin variant *Human hemoglobin β-chain* *2,3-DPG binding*

1. INTRODUCTION

A 67-year-old Scotsman was admitted to Coventry and Warwickshire Hospital with abdominal pain and vomiting. Laparatomy revealed a large mass above and adherent to the duodenum which turned out to be an adenocarcinoma. The patient died shortly afterwards. A blood count using Technicon H6000 had revealed an abnormal distribution of red cell size and an abnormal red cell lysis picture. This caused examination of the haemolysate by electrophoresis and an abnormal band was seen which on further investigation turned out to be a hitherto undescribed human haemoglobin variant.

2. METHODS

The determination of haemotological data followed the established routine [1]. Besides the Coulter Counter, the Technicon H6000 was also used on some occasions. The lability of the haemoglobin was determined by the isopropanol and the zinc tests [2,3]. Procedures for preparation of haemolysates, separation of haemoglobin by paper and cellulose acetate electrophoresis at pH 8.9, quantitation of haemoglobin fractions, preparation of globin, of tryptic peptides derived therefrom, their two-dimensional separation by high-voltage electrophoresis and chromatography, elution of peptides and their analysis have been summarised [4]. Globin chain separation followed established techniques [5,6]. For functional studies the haemoglobin was isolated by electrofocusing [7]. An automatic oxygenation apparatus was used [8] and the data were analysed by a microcomputer [9]. Methaemoglobin was determined using published molecular extinction coefficients [10].

3. RESULTS

The haemolysate of the patient contained 38% of the haemoglobin variant and lability tests were slightly positive after 15 min with isopropanol and after 30 min with zinc. Chain separation on cellulose acetate indicated that the variant had an

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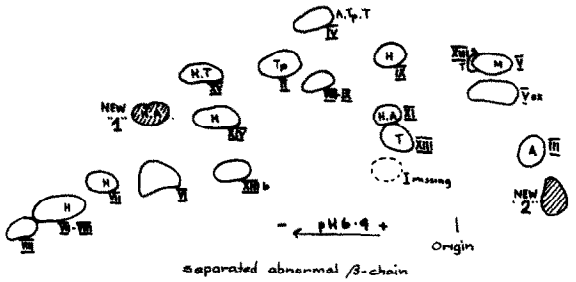

Fig.1. 'Fingerprint' of the tryptic peptides of the β-chain of Hb Warwickshire. Amino acid stains: A, arginine; H, histidine; T, tyrosine; Tp, tryptophan. The tryptic peptide β1 (β1–8) is missing. Two new peptides are seen instead. They correspond to β1–5 and β6–8, respectively. For details see text.

additional positive, or one less negative charge in the β-chain. The β-chain was isolated, digested with trypsin and the tryptic digest was 'fingerprinted'. The two-dimensional chromatogram showed that peptide βTpI (β1–8) was missing and that two new peptides had appeared instead (fig.1). One of these, no.1 in fig.1, stained for histidine and arginine and on analysis contained 5-amino acid residues: 4 of these corresponded to the first 4 residues of βTpI (Val_1, His_1, Leu_1, Ser_1), the fifth amino acid was an arginine and as this was a tryptic peptide it had to be in the C-terminal position. The other peptide (no.2) was on analysis Glu_2, Lys_1. The electrophoretic mobility of this peptide indicated that both 'Glu' residues were derived from glutamic acid rather than from glutamine and that this tryptic peptide corresponded to the last three residues of βTpI: Glu→Glu→Lys. Thus the difference of the β-chain variant from β^A was a change at position β5 [A2] of pro→Arg.

The role of β5 Pro is of interest because the γ-chain has a Glu in that position, and it was decided to submit the new haemoglobin to a careful examination of its functional properties in Dr Imai's laboratory. As will be seen from table 1, the oxygen dissociation curve of Hb A and Hb Warwickshire are practically identical in the absence of phosphates. The curves (not reproduced here) are superimposable. However, there is a small but definite fall in oxygen affinity when Hb Warwickshire is compared with Hb A in the presence of phosphates.

Table 2 shows that the haematological findings in the propositus and his family were normal. The mild instability of the haemolysate found in the

Table 1

Oxygenation properties of Hb A and Hb Warwickshire

Conditions[a]		P_{50} (mmHg)	r[b]	$P_{50}^{abn}/P_{50}^{nor}$	$P_{50}^{phos}/P_{50}^{none}$ (pH 7.4–7.5)	n_{max}[c]	Met Hb% after measurement
pH	Phosphate						
Hb A							
7.9	none	2.44		–	–	2.79	7.3
7.4	none	4.20	0.48	–	–	2.90	10.3
6.95	none	6.68		–	–	2.93	8.4
7.45	1 mM DPG	9.96		–	2.37	3.07	7.2
7.5	1 mM IHP	33.9		–	8.07	2.62	5.4
Hb $\alpha_2\beta_2$ 5Pro→Arg							
7.9	none	2.47		1.01	–	2.93	6.1
7.4	none	4.22	0.48	1.00	–	2.97	8.0
6.95	none	6.87		1.03	–	2.96	7.1
7.45	1 mM DPG	11.0		1.10	2.61	3.05	5.8
7.5	1 mM IHP	39.3		1.16	9.31	2.54	5.4

[a] Other conditions: in 0.5 M Tris buffer (pH 7.9) or 0.05 M bistris buffer (pH 7.4–7.5) containing 0.1 M Cl^-, at 25°C; haemoglobin concentration, 60 μM on haem basis

[b] Bohr coefficient ($-\Delta \log P_{50}/\Delta pH$)

[c] Maximal slope of the Hill plot

Table 2
Haematological findings

Relation to propositus and d.o.b.	Hb (g/dl)	RBC ($\times 10^{12}$/l)	Hct (l/l)	MCH (pg)	MCV (fl)	MCHC (g/dl)	Abnormal (Hb%)	Hb F (%)	HbA_2 (%)
Propositus 1914	12.9	4.60	40.8	28.0	89	31.6	38	0.4	3.2
Daughter 1937	12.9	4.61	39.8	28.0	86	32.4	none	0.5	3.4
Son 1942	14.1	4.46	39.6	31.9	88	35.9	none	0.5	3.2
Son 1945	15.0	5.07	44.2	29.8	87	34.2	31	0.5	2.7
Brother 1923	14.8	4.88	41.6	29.5	85	34.6	36	0.5	3.3

propositus was present in all other carriers of this haemoglobin. The somewhat abnormal findings seen with Technicon H6000 are sometimes associated with abnormal haemoglobins but also sometimes with changes in the blood chemistry. It was relevant therefore that the same observations were also made with the healthy carriers of Hb Warwickshire. The abnormal haemoglobin when present was not found in exactly the same proportion in the different carriers.

4. DISCUSSION

The replacement of $\beta 5$ [A5] Pro by an arginine increased the phosphate binding capacity of the haemoglobin tetramer as if, as it were, $\beta 5$ Pro assists in the off-loading of 2,3-DPG. Dr M.F. Perutz suggested that such observations may throw light on the 'fine tuning' of the 2,3-DPG binding of which of course the major residues involved, $\beta 1$ Val, 2 His, 82 Lys and 143 His, are well recognised. It would be of interest to find substitutions of $\beta 5$ Pro other than Arg and to test whether they also affect the O_2 binding in the presence of phosphates. This would indicate whether the change in off-loading of the 2,3-DPG is due to the absence of $\beta 5$ Pro or the introduction of the positively charged arginine residue; its distance from the DPG is however too far to permit a direct interaction (M.F. Pertuz, personal communication).

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REFERENCES

[1] Dacie, J.V. and Lewis, S.M. (1975) in: Practical Haematology, 4th edn. F.A. Davis, Philadelphia, PA.
[2] Carrell, R.W. and Kay, R.A. (1972) Br. J. Haematol. 23, 615–619.
[3] Carrell, R.W. and Lehmann, H. (1981) J. Clin. Pathol. 34, 796–799.
[4] Lehmann, H. and Huntsman, R.G. (1974) in: Man's Haemoglobins, 2nd end. Elsevier, Amsterdam.
[5] Clegg, J.B., Naughton, M.A. and Weatherall, D.J. (1966) J. Mol. Biol. 19, 91–108.
[6] Ueda, S. and Schneider, R.G. (1969) Blood 34, 230–235.
[7] Harano, T., Iuchi, I. and Shibata, S. (1977) Kawasaki Med. J. 4, 53–56.
[8] Imai, K. (1981) Methods Enzymol. 76, 438–449.
[9] Imai, K. (1982) in: Allosteric Effects in Haemoglobin, Cambridge University Press, Cambridge.
[10] Van Assendelft, O.W. and Zijlstra, W.G. (1975) Ann. Biochem. 69, 43–48.