HAEMOGLOBIN PORT PHILLIP α91 (FG3) LEU→PRO

A new unstable haemoglobin

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1. Introduction

A Chinese female laboratory technician had a mild anaemia, initially thought to be thalassaemia. On further investigation a new abnormal haemoglobin (Hb Port Phillip) was identified with the substitution leucine to proline at residue 91 (FG3) of the α-chain. This mutation will cause the loss of a haem contact which explains the decreased stability of the haemoglobin. There is also a general distortion of the FG corner that should provide a test of currently proposed mechanisms for the co-operative interaction of haemoglobin subunits.

2. Methods

Standard procedures were used for the haematological investigations including haemoglobin stability tests [1] and for the detection, isolation and identification of the abnormal haemoglobin [2]. Tryptic digestion and peptide mapping were carried out as described by Watson-Williams et al. [3]. Quantitation of Hb Port Phillip was by elution from DEAE–Sephadex [4], peak areas were calculated by triangulation. Separation of α- and β-chains and subsequent aminoethylation was carried out as described by Clegg et al. [5].

Digestion with thermolysin was carried out overnight at 37°C. The peptide was dissolved in 1 ml 0.2 M ammonium bicarbonate and 0.1 ml thermolysin (2 mg/ml in water) was added.

3. Results

3.1. *Haematological findings*

The haemoglobin level (10.7 g/100 ml), the mean cell volume (76 fl) and the packed cell volume (34%) were all low (normal values, 12–16 g/100 ml, 80–95 fl and 37–47%, respectively). The reticulocyte count (3–4%) and the HbF (5%) and HbA_2 (3.7%) levels were raised (normal values 0.2–2%, less than 0.8% and 1.8–3.3%, respectively).

Precipitates were obtained from haemolysates with both the heat [1] and isopropanol stability tests [6].

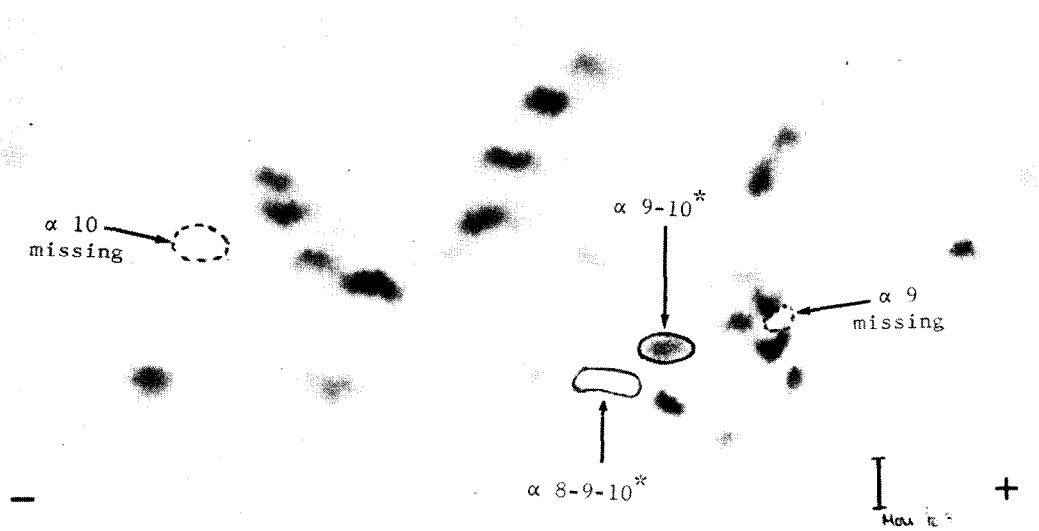

Fig.1. Tryptic peptide map of Hb Port Phillip. Electrophoresis, at pH 6.5, ascending chromatography in pyridine/isoamyl alcohol/water (6:6:7, v/v/v).

The isopropanol precipitate represented 5% of the total haemoglobin. Starch gel electrophoresis, at pH 8.6, indicated the presence of an additional band running in the same position as HbF. A similar slow running HbA_2 band was also present. On chromatography on DEAE–Sephadex [4] Hb Port Phillip ran as a shoulder on the HbA peak whilst the HbF eluted after the HbA. Quantitation showed that the new haemoglobin formed 7% of the total haemoglobin.

3.2. *Structural studies*

The unstable haemoglobin component was isolated by preparative isopropanol precipitation [6]. Starch gel electrophoresis, pH 8.6, in 6 M urea, of the precipitated globin demonstrated that no change was present on either the α- or β-chain.

Tryptic peptide maps indicated that the arginine-containing peptide α10 and the methionine-containing peptide α9 were missing. Two new arginine and methionine-staining spots were present, α9–10* and α8–9–10* (fig.1).

The abnormal α-chain was isolated by chromatography of the isopropanol-precipitated globin on CM-cellulose in 8 M urea [5]. After aminoethylation, tryptic peptide maps were prepared of the abnormal α-chain. These maps confirmed that a new peptide (α9–10*) was present. This peptide was eluted with 0.1 M ammonium hydroxide from four preparative fingerprints. One quarter of the eluted material was taken for amino acid analysis which indicated (table 1) a composition similar to α9 except that two additional residues, proline and arginine, were present.

This evidence suggested a mutation of α91 Leu→Pro. In order to confirm this the remaining peptide material was digested with thermolysin. A thermolytic peptide map appeared normal except

Table 1
Amino acid composition of peptide α9–10* from Hb

Amino acid	Observed molar ratio α9–10*	Expected molar ratio α9	α10
Asp	6.1	6	–
Thr	1.1	1	–
Ser	2.0	2	–
Pro	2.0	1	–
Ala	6.8	7	–
Val	2.5[a]	3	–
Leu	4.5	4	1
Lys	1.2	1	–
His	2.7	3	–
Arg	1.1	0	1

[a] N-Terminus, partially destroyed by reaction with ninhydrin

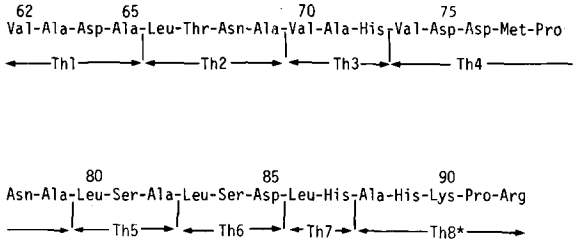

Fig.2. Thermolytic peptides (Th) produced on cleavage of peptides α9–10* from Hb Port Phillip.

that a basic peptide Th8* (fig.2) stained positively for arginine. Peptide Th8* was isolated by preparative peptide mapping and eluted in 6 M hydrochloric acid. Amino acid analysis indicated a composition of, Ala, His, Lys, Pro, Arg, compatible with the electrophoretic mobility of the peptide [7].

These results confirm that this is a new haemoglobin with the substitution α91 (FG3) leucine to proline, the presence of a proline distal to lysine α90 preventing tryptic cleavage and giving rise to the new peptide α9–10.

4. Discussion

This is the first mutation found at residue FG3 in either the α-, β-, γ- or δ-chains of haemoglobin. Both this leucine and its neighbouring residue, valine FG5, form contacts with the haem group and play a critical part in the co-operative effects between subunits that occur on oxygenation [8,9]. The steric situation and proposed role of the α91 leucine are well illustrated by Gelin and Karplus [10]. They propose that this leucine contributes to the tilting of the haem group and the movement of the FG corner that occurs on oxygenation.

The loss of a haem contact and general perturbation that results from the substitution of a proline for the leucine explains the decreased haemoglobin stability and consequent haemolytic anaemia [11]. It will be of interest to study further the structural and functional alterations in relation to co-operative effects since this mutation should provide a test of proposals for the direct transmission of tertiary changes to other subunits through the FG corner [10].

Acknowledgements

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