Hb NEWCASTLE: β92 (F8) His → Pro

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1. Introduction

A new unstable haemoglobin, Hb Newcastle, is described, in which the important haem-linked proximal histidine residue β92 (F8) is replaced by proline. The propositus, who is 67, has a chronic anaemia which has improved slightly after splenectomy, but is otherwise virtually unaffected by the presence of the variant.

2. Methods

Electrophoresis of oxy- and met-haemolysate was carried out as recently described [1]. Hb A_2 was quantitated by cellulose acetate electrophoresis [2] and Hb F by alkali denaturation [3]. Globin electrophoresis in 6 M urea [4] was performed at pH 5.8, 6.5 and 8.9. Isopropanol stability tests were carried out according to Carrell and Kay [5] and DEAE-Sephadex chromatography according to Huisman and Dozy [6]. Ultraviolet and visible absorption spectra of the oxy-forms of purified Hb A and Hb Newcastle were recorded using a Pye Unicam SP800 recording spectrophotometer.

Structural studies were carried out as recently described [1] except that: (a) the first dimension of the 'fingerprinting' of thermolysin peptides was at pH 3.5 (60 V/cm, 1 h), not pH 6.4 and (b) the aminoethylation procedure was modified as follows:

The isolated salt-free β-chain was dissolved to a concentration of 10 mg/ml in urea-saturated 1 M Tris–HCl, pH 8.6, containing a ten-fold excess of dithiothreitol [7] over protein thiol, and incubated for 3 h at room temperature. A ten-fold excess of ethyleneimine [8] over total thiol was then added and allowed to react under nitrogen at room temperature until the nitroprusside test (carried out according to Shotton and Hartley [9]) was negative, whereupon the reaction was stopped by lowering the pH to 4 with acetic acid. The S-2-aminoethylated (AE) β-chains were dialysed exhaustively against distilled water and recovered by freeze-drying.

Peptides containing divalent sulphur and histidine were located using the chloroplatinate and Pauly reagents, respectively [10]. Dansyl-Edman degradation was carried out as previously described [11].

3. Results

The propositus, a 67 year old English woman, had anaemia and reticulocytosis (Hb 7–7.6 g/dl, reticulocytes 18%) with recurrent jaundice. After splenectomy, her haematological parameters improved slightly (Hb 9.3 g/dl, reticulocytes 11%) and 80% of her red blood cells were observed to contain Heinz bodies. Her father, who is dead, had suffered from anaemia and jaundice all his life. The isopropanol stability test produced a small amount of a whitish precipitate, suggesting mild instability and a tendency to lose haem groups; the precipitate at early times consisted almost exclusively of β-chains. Electrophoresis of haemolysate on all media showed the presence of an additional band which migrated between Hb A and Hb A_2; the exact position of the band varied according to the pH, the support medium and whether the haemolysate was in the met- or oxy-form. DEAE-

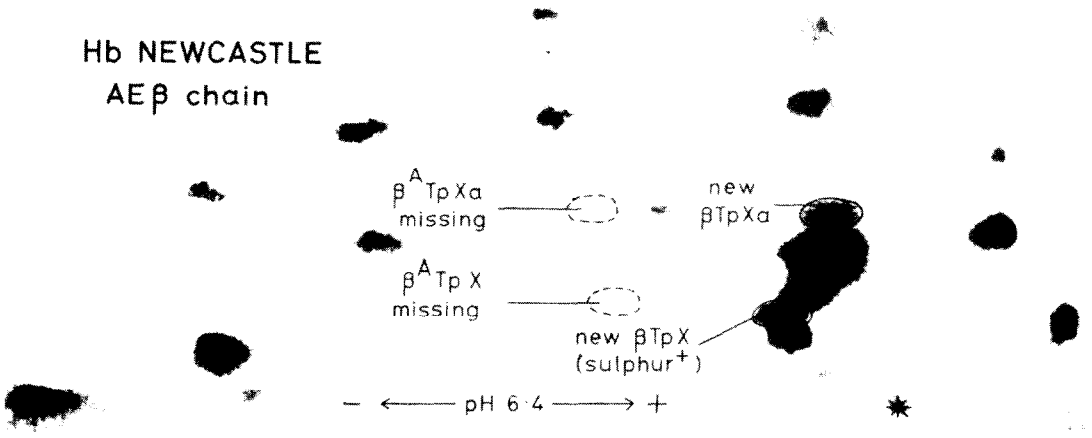

Fig.1. 'Fingerprint' of tryptic peptides of the AEβ chain from Hb Newcastle, prepared as described in text. (*) = Origin.

Sephadex chromatography showed the abnormal component to elute shortly after Hb A_2, at a pH value of 7.95 (Hb A_2 and Hb A eluted at values of 8.10 and 7.60, respectively). The absorption spectrum of the oxy-form of the purified variant showed the absorbance ratios 540/280 nm and 412/280 nm to be reduced relative to those of Hb A, suggesting a loss of haem groups. The level of Hb A_2 was 3.6%, which is just above the normal range (2.5–3.5%), while the Hb F level was elevated to 3.6%. The proportion of the variant, estimated after cellulose acetate electrophoresis and not corrected for haem loss, was 17%. Electrophoresis of globin from the haemolysate in 6 M urea showed no abnormality at pH 6.5 or at pH 8.9, but a fast-migrating β-chain was observed at pH 5.8. The CM-cellulose elution profile of such globin showed no abnormality at pH 6.73, but at pH 6.21 the variant β-chains eluted between the normal γ- and β-chains. This behaviour would be consistent with the replacement of a histidine residue by a neutral residue.

Integration of the peak areas after CM-cellulose chromatography at pH 6.21 showed the variant β-globin chains to constitute 26% of the total non-α-chains which is presumably a more accurate estimate of the proportion of the variant than that obtained by cellulose acetate electrophoresis of the whole haemoglobin. The 'fingerprint' of the tryptic peptides from the variant AEβ chains (fig.1) showed the

Table 1
Hb Newcastle; amino acid compositions of the tryptic peptides βX and βXa.

Amino acid	Molar ratios			
	βX	Expected for β^AX	βXa	Expected for β^AXa
Asp	1.04	1	–	–
Thr	1.88	2	1.76	2
Ser	0.91	1	0.93	1
Glu	1.18	1	1.09	1
Pro	0.90	–	0.98	–
Gly	0.86	1	1.21	1
Ala	0.93	1	1.15	1
Leu	2.03	2	2.00	2
Phe	1.00	1	0.98	1
His	–	1	–	1
Lys + AECys	1.94	2	0.76 (AECys)	1

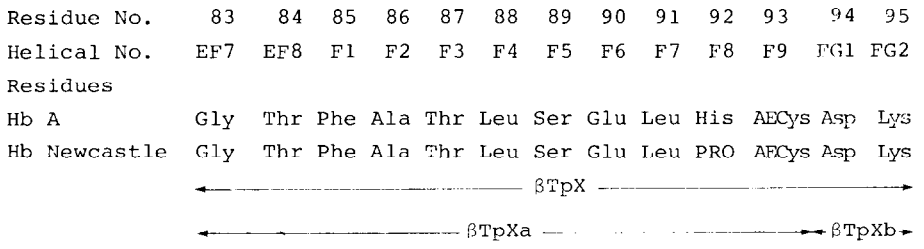

Fig.2. The amino acid sequence of βTpX from Hb A and Hb Newcastle.

absence of βTpX (β83–95) and βTpXa (β 83–93), both of which normally give positive staining reactions for divalent sulphur and histidine, and the presence of a positive divalent sulphur stain in the neutral region. Electrophoresis of the latter at pH 3.5 showed the presence of two new peptides, the amino acid compositions of which are shown in table 1. It is clear from these compositions that the single histidine residue normally found in βTpX, at position β92 (see fig.2), has been replaced by proline. This was substantiated by the isolation and sequencing of the abnormal thermolysin peptide Leu–Ser–Glu–Leu–Pro–(AECys, Asp, Lys) (β88–95, see fig.2). Thus the variant, which was named Hb Newcastle, is β92 (F8) His → Pro.

4. Discussion

Replacement of the proximal histidine βF8 should have repercussions for the binding of haem to the variant β-chains. When this residue is replaced by glutamine, as in Hb St Etienne [12] (Istanbul [13]), the haemoglobin is incapable of binding haem to the abnormal chains [12] and behaves as a natural semi-haemoglobin [14,15]. This may be relevant for Hb Newcastle, which shows low 540/280 nm and 412/280 nm absorbance ratios, compatible with loss of haem from the β-chains. It has, however, yet to be determined whether or not the loss of haem groups is an artefact due to the isolation methods. It is of some interest that the same substitution (His → Pro) at the distal histidine (β63 E7) does not result in the loss of haem groups from the abnormal chains [16].

The instability of Hb Newcastle probably results from a combination of loss of haem groups (which help to stabilise the subunit tertiary structure [17]) and the introduction of a proline residue into the middle of an α-helix; the implications of the latter have been discussed elsewhere [18].

When more material becomes available, functional and biosynthetic studies are planned.

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