THE ζ-CHAIN, AN α-LIKE CHAIN OF HUMAN EMBRYONIC HAEMOGLOBIN

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1. Introduction

Haemolysate from a hydropic infant, who had died immediately after delivery at 32 weeks, showed on paper electrophorises at pH 8.9 [1], the features of Hb Bart's hydrops foetalis. Most of the haemoglobin was in the position of Haemoglobin Bart's (γ_4) and a small fraction amounting to less than 10% was seen in the position of Hb A, now known in these cases to be not Hb A but Hb Portland ($\zeta_2\gamma_2$) [2–4].

2. Materials and methods

For structural study, the whole haemolysate was freed from haem with HCl–acetone at 20°C [5], and the precipitated globin thoroughly washed with cold acetone and dried under nitrogen. Aminoethylation of the globin was carried out in 8 M urea at pH 9.1 for 2 hr [6] and recovered after thorough dialysis against 0.5% formic acid and freeze drying. 8.2 mg of the aminoethylated globin was hydrolysed with trypsin

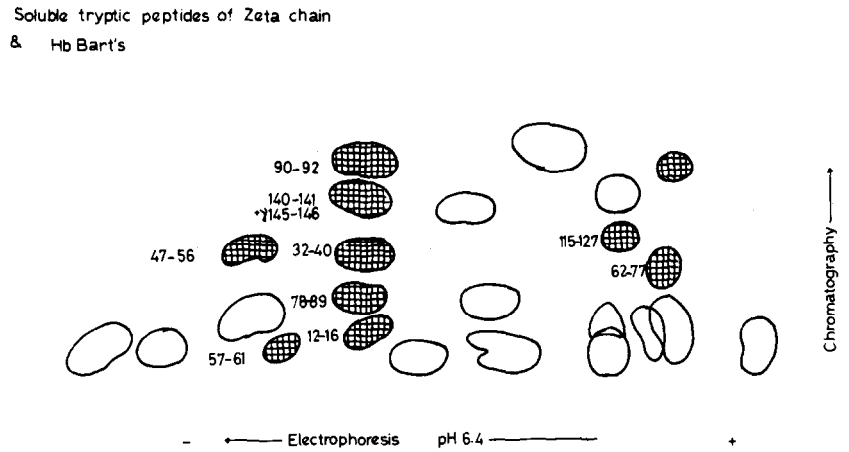

Fig. 1. Fingerprint of the soluble tryptic peptides of the haemoglobin of the patient. The shaded peptides are considered to belong to the ζ-chain.

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(TPCK treated, Worthing Biochemical Corp., N.J., USA) at pH 8.6 for 2 hr. The soluble tryptic digest was applied to 2 Whatman 3MM papers for two dimensional peptide mapping [7]. The peptides were identified either by staining with 0.02% ninhydrin in acetone or with fluorescamine [8] 'Fluram' F. Hoffmann – La Roche and Co., A. G. Diagnostica, Basel, Switzerland (1 mg fluorescamine/100 ml dry acetone diluted 10 fold with 10% pyridine in dry acetone). The peptides were further purified by paper electrophoresis at pH 3.5 or 9.0, eluted and hydrolysed in constant boiling 6N HCl for 24 hr in sealed capillary tubes, dried and then applied on a Locarte Amino acid Analyser for determination of their amino acid composition [9].

3. Results

The soluble tryptic peptides were prepared from the whole haemolysate consisting of Hb Bart's (γ_4) and Hb Portland ($\zeta_2\gamma_2$). The fingerprint which was stained with ninhydrin showed γ-chain peptides only. Additional peptides were seen when the second fingerprint was stained with fluorescamine. These additional faint peptides were considered to belong to the ζ-chain (fig. 1). The amino acid compositions of these peptides (table 1) were determined and found to have some resemblance to α-chain peptides. In fig. 2 these peptides are aligned with the α-chain and the peptides (tryptic and one chymotryptic) obtained by Capp, Rigas and Jones using a different technique [10]. Except for the position of the C-terminus of the tryptic peptides, all residues are placed by homology on alignment with the human α-chain, and no sequencing of residues was possible because of the shortage of the available material.

Table 1
ζ-Chain Peptides. Amino Acid Composition in molar ratios

Aligned with α12–16 (fluoresced under UV light)		
Aspartic Acid	1.2	(1)
Glycine	1.0	(1)
Alanine	1.0	(1)
Lysine	1.0	(1)
Tryptophan	++	
Yield/residue	9.5 nmoles	

Aligned with α32–40		
Threonine	1.0	(1)
Serine	1.0	(1)
Glutamic Acid	1.1	(1)
Proline	0.8	(1)
Leucine	2.1	(2)
Phenylalanine	1.2	(1)
Histidine	0.6	(1)
Lysine	1.1	(1)
Yield/residue	8.4 nmoles	

Aligned with α47–56		
Threonine	0.7	(1)
Glycine	1.2	(1)
Alanine	1.0	(1)
Valine	1.0	(1)
Leucine	2.0	(2)
Histidine	2.1	(2)
Lysine	1.1	(1)
Yield/residues	8.5 nmoles	

Aligned with α57–61		
Serine	0.6	(1)
Glycine	1.2	(1)
Alanine	1.3	(1)
Histidine	0.7	(1)
Lysine	1.2	(1)
Yield/residues	6.8 nmoles	

Aligned with α62–77		
Aspartic acid	1.4	(1)
Threonine	1.0	(1)
Serine	2.0	(2)
Glutamic acid	1.1	(1)
Proline	1.0	(1)
Glycine	1.4	(1)
Alanine	2.1	(2)
Valine	2.1	(2)
Isoleucine	0.7	(1)
Phenylalanine	0.8	(1)
Lysine	0.9	(1)
Yield/residue	5.0 nmoles	

Aligned with α78–89		
Aspartic acid	0.8	(1)
Serine	0.6	(1)
Glutamic acid	0.5	(1)
Glycine	1.0	(1)
Alanine	2.9	(3)
Valine	1.9	(2)
Histidine	0.7	(1)
Lysine	1.1	(1)
Yield/residue	8.8 nmoles	

Aligned with α90–92	
Isoleucine	1.1 (1)
Leucine	1.2 (1)
Arginine	0.7 (1)
Yield/residue	10.27 nmoles

Aligned with α115–127	
Aspartic acid	1.7 (2)
Threonine	0.9 (1)
Glutamic acid	1.0 (1)
Glycine	1.3 (1)
Alanine	2.0 (2)
Valine	1.4 (1)
Isoleucine	0.9 (1)
Leucine	1.5 (1) or (2)
Phenylalanine	1.0 (1)
Lysine	0.9 (1)
Yield/residue	6.9 nmoles

One peptide had the following composition:

	Yield
Tyr	66 nmoles
Histidine	110 nmoles
Arginine	5.2 nmoles

This was presumed to be a mixture of Tyr–His from the γ-chain and Tyr–Arg from the ζ-chain.

In addition the following peptide was isolated in the γ^{9b} (γ77–92) region. It was presumed to be a mixture of γ^{9b} (Asp_2 Leu_2 His Lys) with another peptide running with it, presumably of the composition Thr_2 Ser Glu_2 Pro Gly Ala_2 Ile Phe Lys.

Aspartic Acid	20.6 (nmoles)
Threonine	15.2
Serine	7.6
Glutamic Acid	17.3
Proline	9.2
Glycine	9.8
Alanine	17.0
Isoleucine	4.3
Leucine	21.7
Phenylalanine	9.2
Histidine	9.7
Lysine	20.7

In previous work a rather similar kind of peptide was observed with a composition Lys Thr_2 Ser Gly,Ala_2 Leu. On the basis of the amino acid compositions, these peptides could not be aligned with any of the known α-chain peptides.

4. Discussion

The ς-chain is a distinct human globin chain which is synthesised during embryonic development. The composition of the tryptic peptides of the ζ-chain are impressively similar to those of the α-chain. Alignment of all but one peptide isolated from the tryptic hydrolysate is possible as shown in Fig. 2. Residues which can be aligned to be fully alike are shown in boxes. For others alignment attempts to maximise similarity of polarity and size, and also takes into account the position of haem contacts, and the intra molecular contacts of the α and β chains according to Perutz's model [11] as applied to man. The ζ-chain clearly resembles the α-chain more than the non α-chains. The tryptic dipeptide Tyr-Arg isolated from the ζ-chain is characteristic of all α-chains and may represent the C-terminal residues of the ζ-chain. In addition to the possibility of marked similarities in the C-terminal region of the α-chain and the ζ-chain, important identities appear to exist in the region of CD6-E5 (α48–56) which is highly conserved in known mammalian α-chains but different on the non α-chains. Two residues of the ζ-chain can be aligned with invariant α-chain residues in the region between αCD8 and αE2 (α53 and α55). None of the tryptic peptides isolated from the ζ-chain so far are similar to the seven residues which account for the D-helix present only in the non α-chains of haemoglobin. Of 28 internal residues available for comparison 20 are alike, 7 very similar, and only 1 dissimilar to those of the α-chain (α33). Of 12 haem contacts available for comparison 9 are the same in the alignment as those of the α-chain, notably B13 which is Met in the α-chain but Leu in the β-chain. As regards the subunit contacts, of the 16 α1β1 contacts the alignment fails to accommodate identical residues only at 5 positions: α106 Leu/Gly, 107 Val/Lys, 114 Pro/Tyr, 119 Pro/Ile, and 122 His/Asx. Of the 5 α1β2 contacts available all can be aligned to be identical with the α-chain. In certain of these residues the α- and β-chains are invariably different. They are in the α-chain and in the aligned ζ chain at positions 37, 38, 40, 91 and 92: Pro, Thr, Lys, Leu, Arg respectively, whilst the corresponding β-chain residues are Pro, Trp, Gln, Leu and His. These probable similarities in the structure of the α- and ζ-chains indicate that the latter might possibly form co-operative tetramers with non α-chains. These may be necessary for normal

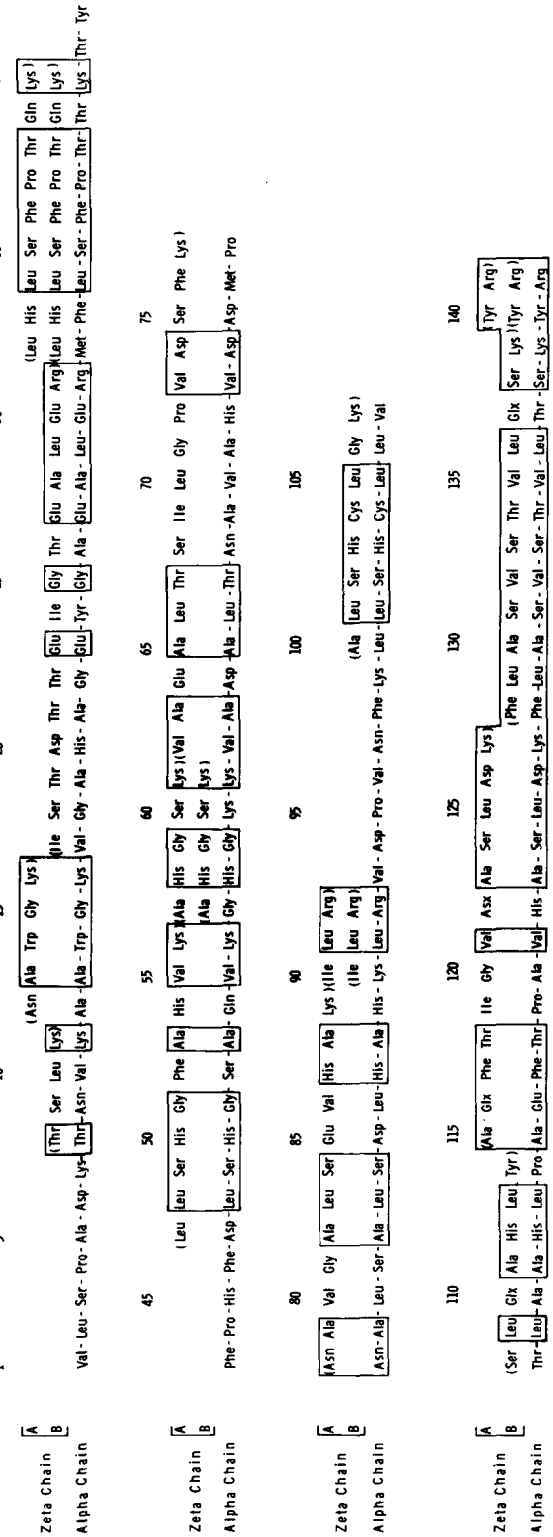

Fig. 2. Alignment by homology of ζ-chain peptides with the α-chain of human haemoglobin (A – present paper, B – ref. [10]). Note that as in the α chain there are no residues corresponding to the D Helix and that there is good correspondence from α50 (CD8) to α53 (E2).

embryonic development. In homozygous α-thalassaemia, (Bart's hydrops foetalis syndrome) complete suppression of α-chain production may be due to failure of switching over from ζ- to α-chain synthesis. Failure to produce α-chains causes the non α-chains to form tetramers of like chains: β_4, γ_4. In contrast to tetramers composed of two pairs of unlike chains, β_4 and γ_4 show no co-operative oxygen binding. Only the α- and the ζ-chain have been found capable of forming tetramers with pairs of other chains [3,4]. Possibly the continuation of ζ-chain production in small though measurable amounts found in homozygous α-thalassaemia explains why the affected infants can survive in utero at all and sometimes even for a few hours after birth.

References

[1] Cradock-Watson, J. E., Fenton, J. C. B. and Lehmann, H. (1959) J. Clin. Pathol. 12, 372.

[2] Capp, G. L., Rigas, D. A. and Jones, R. T. (1967) Science 157, 65.

[3] Weatherall, D. J., Clegg, J. C. and Boon, W. H. (1970) Brit. J. Haematol. 18, 357.

[4] Todd, D., Lee, M. C. S., Beaven, G. H. and Huehns, E. R. (1970) Brit. J. Haematol. 19, 27.

[5] Rossi-Fanelli, A. R., Antonini, E. and Caputo, A. (1958) Biochim. Biophys. Acta 30, 608.

[6] Jones, R. T. (1964). Cold Spring Harbor Symposium on Quantitative Biology. 29, 297.

[7] Sick, K., Beale, D., Irvine, D., Lehmann, H., Goodall, P. T. and MacDougall, S. (1967) Biochim. Biophys. Acta, 140, 231.

[8] Udenfriend, S., Stein, S., Böhlen, P., Dairman, W., Leimgruber, W. and Weigekem, M. (1972) Science 178, 871.

[9] Spackman, D. H., Stein, W. H. and Moore, S. (1958) Analyt. Chem. 30, 7, 1190.

[10] Capp, G. L., Rigas, D. A. and Jones, R. T. (1970) Nature 228, 278.

[11] Perutz, M. F. (1969) Proc. Roy. Soc. B. 173, 113.