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Amino acid sequence of the fetal chain of yak haemoglobin

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Abstract. The amino acid sequence of the fetal chain of yak haemoglobin was determined. The sequence is the same as that of the fetal chain of bovine haemoglobin. Phenylalanine is present at position 12 of the helix A in the fetal chain while tryptophan is the amino acid at this position in the β-chain of yak adult haemoglobin. This amino acid replacement may be responsible for the higher oxygen affinity of yak fetal haemoglobin than yak adult haemoglobin.

Keywords. Yak; fetal haemoglobin; amino acid sequence; oxygen affinity.

Introduction

Fetal haemoglobins which differ from the corresponding adult haemoglobins in their non-α chains are found in different species of Bovidae during fetal development (Kitchen and Brett 1974; Wilson *et al.,* 1966). The fetal haemoglobins normally have higher oxygen affinities than their adult counterparts (Barron, 1951; Hellegers *et al.,* 1959; Bard and Shapiro, 1979; Metcalfe *et al.,* 1967). In man the difference in the oxygen affinities of the adult and the fetal haemoglobins is due to the difference in their degree of interaction with 2,3-diphosphoglycerate (2,3-DPG), the allosteric modulator of oxygen affinity (Bauer *et al.,* 1968, 1969). The oxygen affinities of bovid haemoglobins are not much affected by 2,3-DPG (Bunn, 1971) and the mechanism of regulation of oxygen affinities of these haemoglobins is yet unknown. The structural basis for the difference in the oxygen affinities of bovine fetal and adult haemoglobins has been proposed by Perutz and Imai (1980). In order to find out whether this is true for other species of Bovidae, we have determined the amino acid sequence of the fetal chain of yak haemoglobin.

Materials and methods

Blood was obtained by venepuncture from a month-old calf from Hellabrunn Zoo, Munich, using heparin as anticoagulant. The erythrocytes were separated by centrifugation and washed 3 times with physiological saline. They were then lysed with an equal volume of distilled water in the presence of 0·4 volume of toluene. Globin was prepared by acid-acetone precipitation method (Rossi-Fanelli and Antonini, 1958). The different polypeptide sub-units were identified by polyacrylamide gel electrophoresis in the presence of 8 M urea and Triton X-100 (Alter *et al.,* 1980). The globin chains were isolated by ionexchange chromatography (Clegg *et al.,* 1966).

Twenty-five mg of the isolated polypeptide chain was digested with trypsin after

Abbreviations used: 2,3-DPG, 2,3-Diphosphoglycerate; HPLC, high pressure liquid chromatography.

performic acid oxidation (Hirs *et al.,* 1956) or S-carboxymethylation (Crestfield *et al*., 1963). Fragmentation of the polypeptide chains was also achieved by acid cleavage (Jauregui-Adel and Marti, 1975). The large peptides obtained by chemical cleavage were fractionated by gel filtration on Sephadex G-50 using 8 M urea-10% formic acid as the eluent. The tryptic peptides were fractionated by high pressure liquid chromatography (HPLC) on Lichrosorb RP-2 (Merck, Darmastadt) using 50 mM ammonium acetate buffer at pH 6·5 applying a linear gradient of 0–60% acetonitrile over a period of 60 min. Some factors obtained from HPLC were further resolved by thin layer electrophoresis on cellulose acetate plates using 5% pyridine-acetic acid buffer at pH 5·2 containing 5% acetone.

An average yield of approximately 60% of the tryptic peptides was obtained (the yield varies from one peptide to the other depending on the number of steps required for its purification). The peptides were hydrolyzed in 5·7 N. HCl at 110° for 20 h and the amino acid analyses were performed on Biotronic amino acid analyzer LC 5000. Tryptophan was estimated after hydrolysis of the peptides in the presence of 6% thioglycollic acid. The amino acid sequences were determined with Beckman sequencer Model 890B and 890C by automatic Edman degradation (Edman and Begg, 1967) using Quadrol and N,N'-diethylaminopropyne programmes (Edman and Begg, 1967; Braunitzer and Schrank, 1970). The amino acids were identified as their phenylthiohydrantoin derivatives by thin layer chromatography on Silica plates using the solvent system of Braunitzer *et al.* (1978) and by HPLC (Zimmerman and Pisano, 1977; Lottspreich, 1980) using an apparatus from Hewlett Packard (HP 1084).

The peptides were aligned by comparing with the sequence of fetal chain of cow haemoglobin. The positions of free lys and arg were assigned by homology with the sequence of the fetal chain of cow haemoglobin. Moreover, small quantities of T– 8 + 9 and T – 14 + 15 due to non cleavage of lys-lys and Arg-Arg peptide bonds respectively were obtained.

Results and discussion

The total globin of the blood sample obtained had 4 polypeptide chains all of which can be resolved by polyacrylamide gel electrophoresis in the presence of urea and Triton X-100 as well as by ion exchange chromatography. From their electrophoretic mobilities and elution pattern in ion exchange chromatography, two of the polypeptide chains correspond to the two *α*-chains and one polypeptide chain corresponds to the *ß*-chain of yak adult haemoglobin. One polypeptide chain, the fetal chain, is distinct from the polypeptide chains of adult haemoglobin. Thus the adult and fetal haemoglobins of yak have identical α-chains, while they differ in their non-*α* chains.

Acid cleavage of the fetal chain yielded two fragments which could be fractionated by gel filtration on Sephadex G-50. On fractionation of the tryptic peptides by HPLC (figure 1) followed by thin layer electrophoresis 18 peptides were obtained. The amino acid compositions of the tryptic peptides are given in table 1. Sequence determination was done on the intact chain for the first 40 amino acids using Quadrol program and on the tryptic peptides and the C-terminal acid-cleaved fragment using diethylaminopropyne program for the remaining sequence. Tp-11 had glutamine as the N-terminal amino acid which cyclized into pyroglutamic acid

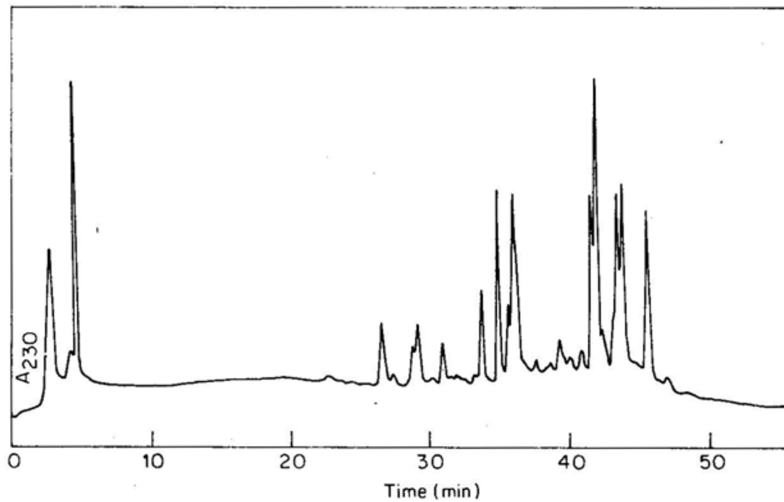

Figure 1. Separation of tryptic peptides of yak haemoglobin fetal chain by HPLC.

thus blocking the free amino group, if allowed to stand for a long time after isolation of the peptide. However, the cyclization did not take place if the sequence determination was done immediately after isolation of the peptide.

The sequence of the fetal chain of yak haemoglobin is given in figure 2. The sequence is identical to that of the fetal chain of bovine haemoglobin (Babin *et al.*, 1966). This is not surprising in view of the fact that yak and cow are closely related and that the sequences of the β^{I} chain of yak haemoglobin (Lalthantluanga *et al.*, 1985) and the β^{A} chain of bovine haemoglobin (Schimenti and Duncan, 1984) are also identical.

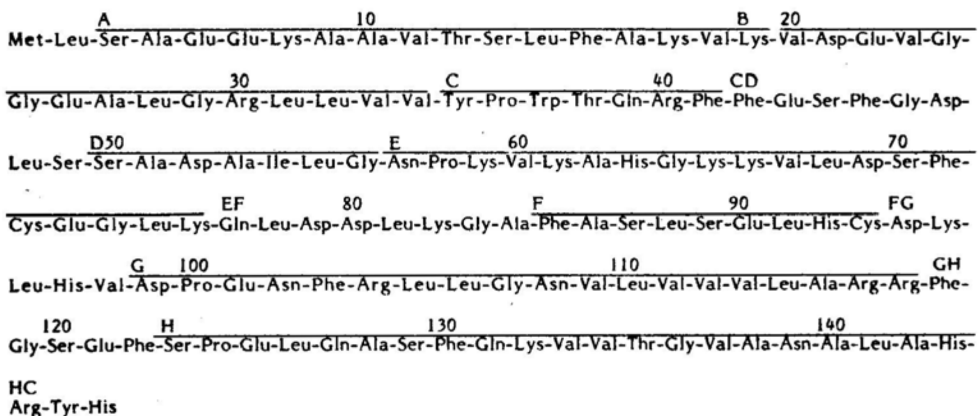

Figure 2. Amino acid sequence of yak haemoglobin fetal chain. The helical segments are indicated by capital letters, A, B, C etc. There is deletion of one amino acid at the amino terminal region of the non-α chains of bovid haemoglobins. A number is given to this deleted residue for the purpose of homology with non-α chains of other mammalian haemoglobins.

Table 1. Amino acid composition of the tryptic peptides of yak haemoglobin fetal chain.

	T-1	T-2	T-3	T-4	T-5	T-6	T-7	T-8	T-9	T-10	T-11	T-12	T-13	T-14	T-15	T-16	T-17	T-18	Total
Asp				1·0		3·0				1·0	2·1	1·0	1·9	1·2			1·0		12
Thr		0·7			0·8												0·9		3
Ser	0·9	0·8				2·6				0·9		1·6				2·6			11
Glu	2·0			1·8	1·1	1·0				1·0	1·1	0·9	0·9			3·9			14
Pro					0·8	1·0							0·9			0·9			4
Gly				2·6		2·0		1·0		1·1		1·0		1·3		1·2	1·2		11
Ala	0·9	2·8		1·0		2·0		1·1				1·8		1·2		1·2	2·9		15
Val		0·9	1·1	1·7	1·9		1·1			0·9			0·9	3·8			2·5		16
Met	0·8																		1
Leu	1·2	1·1		1·2	2·1	2·3				2·1	2·3	2·0	0·9	3·9		1·4	1·4		20
Tyr					0·8													0·9	2
Phe		1·2				2·9				1·0		0·9	1·0			2·9			10
Trp					0·7														1
His								0·9				0·8	0·9				1·0	1·0	5
Lys	1·0	0·9	1·0			1·0	1·0	1·1	1·0	1·0	1·0	1·0				1·1			11
Arg				0·9	0·9								1·0	1·1	1·0		1·0		6
Cys										1·1		1·0							2
Ile						0·9													1
Total	7	9	2	11	10	19	2	4	1	10	6	13	9	12	1	15	12	2	145

The oxygen affinity of the yak fetal haemoglobin is higher than that of bovine fetal haemoglobin (unpublished results). Since the two haemoglobins have identical fetal chains, the difference in their oxygen affinities must be due to the difference in their α-chains. Also, the yak fetal haemoglobin has much higher oxygen affinity than the adult haemoglobin (unpublished results). Perutz and Imai (1980) have suggested that the difference in the oxygen affinities of the bovine adult and fetal haemoglobins may be due to a replacement of Trp A 12 in the *β*-chain by Phe in the fetal chain. This replacement may lower the stability of the tertiary deoxy structure by loosening the contacts between helices A and E and thus increase the oxygen affinity of fetal haemoglobin. This may be the mechanism responsible for the difference in the oxygen affinities of the yak fetal and adult haemoglobins also because Trp and Phe are found at this position in the *β*- and fetal chains respectively of yak haemoglobins.

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References

Alter, B. P., Goff, S. C, Effremov, G. D., Gravely, M. E. and Huisman, T. H. J. (1980) *Br. J. Haematol.,* **44,** 527.
Babin, D. R., Schroeder, W. A., Shelton, J. R., Shelton, J. B, and Robberson, B. (1966) *Biochemistry,* **5,** 1297.
Bard, H. and Shapiro, M. (1979) *Pediat. Res.,* **13,** 167.
Barron, D. H. (1951) *Yale J. Biol. Med.,* **24,** 169.
Bauer, C, Ludwig, M. and Ludwig, J. (1968) *Life Sci.,* **7,** 1339.
Bauer, G, Ludwig, M., Ludwig, J. and Bartels, H. (1969) *Resp. Physiol.,* **7,** 271.
Braunitzer, G. and Schrank, B. (1970) *Hoppe-Seyler's Z. Physiol. Chem.,* **352,** 417.
Braunitzer, G., Schrank, B., Stangl, A. and Scheithauer, U. (1978) *Hoppe-Seyler's Z. Physiol. Chem.,* **359,** 137.
Bunn, H. F. (1971) *Science,* **172,** 1049.
Clegg, J. B., Naughton, M. A. and Weatherall, D. J. (1966) *J. Mol. Biol.,* **19,** 91.
Crestfield, A. M., Moore, S. and Stein, W. H. (1963) *J. Biol. Chem.,* **238,** 622.
Edman, P. and Begg, G. (1967) *Eur. J. Biochem.,* **1,** 80.
Gross, E. and Witkop, B. (1961) *J. Am. Chem. Soc.,* **83,** 15110.
Hellegers, A. E., Meschia, G., Prystowsky, H., Wolkoff, A. S. and Barron, D. H. (1959) *Q. J. Exp. Physiol.,* **44,** 215.
Hirs, C. H. W., Moore, S. and Stein, W. H. (1956) *J. Biol. Chem.,* **219,** 623.
Jauregui-Adel, J. and Marti, J. (1975) *Anal. Biochem.,* **69,** 468.
Kitchen, H. and Brett, I. (1974) *Ann. N. Y. Acad. Sci.,* **241,** 653.
Lalthantluanga, R., Wiesner, H. and Braunitzer, G. (1985) *Biol: Chem. Hoppe-Seyler,* **366,** 63.
Lottspreich, F. (1980) *Hoppe-Seyler's Z. Physiol. Chem.,* **361,** 1829.
Metcalfe, J., Bartels, H. and Moll, W. (1967) *Physiol. Rev.,* **47,** 782.
Perutz, M. F. and Imai, K. (1980) *J. Mol. Biol.,* **136,** 183.
Rossi-Fanelli, A. and Antonini, E. (1958) *Biochem. Biophys. Acta,* **30,** 608.
Schimenti, J. C. and Duncan, C. H. (1984) *Nucleic Acids Res.,* **12,** 1641.
Wilson, J. B., Edwards, W. C, Mc-Daniel, M., Dobbs, M. M. and Huisman, T. H. J. (1966) *Arch. Biochem. Biophys.,* **115,** 385.
Zimmermann, C. L. and Pisano, J. J. (1977) *Methods Enzymol.,* **47,** 45.