THE β CHAIN OF FROG HEMOGLOBIN (*RANA ESCULENTA*): AMINO ACID SEQUENCE OF RESIDUES 35 TO 98

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The α and β chains of the major hemoglobin of the frog *Rana esculenta* have been separated by countercurrent distribution [1,2] and the amino acid sequence of the first 34 residues of the β chain has been established [3]. This paper describes the determination of the sequence of the next 64 residues.

The carboxymethylated [4] and trifluoroacetylated [5] β chain has been subjected to tryptic hydrolysis and five fragments TF_I, TF_{II}, TF_{III}, TF_{IV} and TF_V were produced by cleavage at the 4 arginine residues [3]. These fragments were purified by gel filtration and paper electrophoresis. The peptides TF_I and TF_{II} account for the *N*-terminal part of the chain [3]. The *C*-terminal sequence of TF_{II} is Thr-Gln-Arg and the *N*-terminal sequence of TF_{III} is Tyr-Phe; because a pentapeptide Thr-Gln-Arg-Tyr-Phe was isolated in the chymotryptic digest of the carboxymethylated β chain, TF_{III} could be recognized as the third trifluoroacetylated fragment of the chain.

The polypeptide TF_{III} contains 64 residues, six of which are lysine. After removal of the trifluoroacetyl groups [5], trypsin cleaved this fragment giving 7 peptides (enzyme/substrate weight ratio 1/100; 0.1 M ammonium bicarbonate pH 8.0, 3 hr, 37°). These seven tryptic units could also be identified in the tryptic digest of the non-trifluoroacetylated β chain. They are called T_5 to T_{11} because of their position in the chain. They are purified by paper chromatoelectrophoresis [6] and the amino acid compositions are determined according to Spackman et al. [7]. The sequence of each peptide is established mainly by Edman degradation [8], applied directly on tryptic units or on peptides yielded by chymotryptic cleavage (0.1 M ammonium bicarbonate pH 8.0, 3 hr, 37°) of these units. Table 1 gives the amino acid sequence of the 7 peptides of TF_{III}.

For determining the alignment of the tryptic units, chymotryptic hydrolysis of the polypeptide TF_{III} was performed and the resulting peptides were purified by paper chromatoelectrophoresis. Six chymotryptic peptides ($TF_{III}Ch_1$ to $TF_{III}Ch_6$) allowed placement of the tryptic units in the chain (cf. table 2).

The results so far obtained determine the sequence of the first 98 residues of the β chain, i.e. about 70% of the whole chain. When this part of the molecule is compared to the homologous sequence of the human β chain, it can be noted that 41 out of 98 sites are substituted. The percentage of substitutions (42%) for this section of the frog β chain is much greater than those observed for the complete β chains of mammalian species (0.7% to 23%) [9]. On the other hand the percentage of substitutions is not lower if the γ chain of human fetal hemoglobin, which is supposed to be more primitive than the β chain [10], is taken as reference. But because it is known that during the metamorphosis of tadpoles, the larval hemoglobin is replaced by adult hemoglobin, each with two types of chain none of which being in common [11], it should be of interest to compare the β chain of a tadpole hemoglobin with the human γ chain.

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Table 1
Sequence of tryptic units of the fragment TF_{III}.

Peptide	Sequence	Number of residues
T_5	35 … 53 $\overrightarrow{\text{Tyr}}$ - $\overrightarrow{\text{Phe}}$ - $\overrightarrow{\text{Thr}}$ - $\overrightarrow{\text{Thr}}$ - $\overrightarrow{\text{Phe}}$ - $\underrightarrow{\overrightarrow{\text{Gly}}}$ - $\underrightarrow{\overrightarrow{\text{Asn}}}$ - $\underrightarrow{\text{Leu}}$ - $\underrightarrow{\text{Gly}}$ - $\underrightarrow{\text{Ser}}$ - $\underrightarrow{\text{Ala}}$ - $\underrightarrow{\text{Asp}}$ - $\underrightarrow{\text{Ala}}$ - $\underrightarrow{\text{Ile}}$ - $\underrightarrow{\text{Cys}}$ - $\underrightarrow{\text{His}}$ - $\underrightarrow{\text{Asn}}$ - $\underrightarrow{\text{Ala}}$ - $\underrightarrow{\text{Lys}}$ ←T_5Ch_1→←T_5Ch_2→←T_5Ch_3→←T_5Ch_4→	19
T_6	54 … 60 $\overrightarrow{\text{Val}}$ - $\overrightarrow{\text{Leu}}$ - $\overrightarrow{\text{Ala}}$ - $\overrightarrow{\text{His}}$ - $\overrightarrow{\text{Gly}}$ - $\overrightarrow{\text{Glu}}$ - Lys ←T_6Ch_1→←T_6Ch_2→	7
T_7	61 … 70 $\overrightarrow{\text{Val}}$ - $\overrightarrow{\text{Leu}}$ - $\overrightarrow{\text{Ala}}$ - $\overrightarrow{\text{Ala}}$ - $\overrightarrow{\text{Ile}}$ - $\overrightarrow{\text{Gly}}$ - $\overrightarrow{\text{Glu}}$ - $\overrightarrow{\text{Gly}}$ - $\overrightarrow{\text{Leu}}$ - Lys ←T_7Ch_1→←T_7Ch_2→	10
T_8	71 … 76 $\overrightarrow{\text{His}}$ - $\overrightarrow{\text{Pro}}$ - $\overrightarrow{\text{Glu}}$ - $\overrightarrow{\text{Asn}}$ - $\overrightarrow{\text{Leu}}$ - Lys	6
T_9	77 … 81 $\overrightarrow{\text{Ala}}$ - $\overrightarrow{\text{His}}$ - $\overrightarrow{\text{Tyr}}$ - $\overrightarrow{\text{Ala}}$ - Lys ←T_9Ch_1→←T_9Ch_2→	5
T_{10}	82 … 89 $\overrightarrow{\text{Leu}}$ - $\overrightarrow{\text{Ser}}$ - $\overrightarrow{\text{Glu}}$ - $\overrightarrow{\text{Tyr}}$ - $\overrightarrow{\text{His}}$ - $\overrightarrow{\text{Ser}}$ - $\overrightarrow{\text{Asn}}$ - Lys ←$T_{10}Ch_1$→←$T_{10}Ch_2$→	8
T_{11}	90 … 98 $\overrightarrow{\text{Leu}}$ - $\overrightarrow{\text{His}}$ - $\overrightarrow{\text{Val}}$ - $\overrightarrow{\text{Asp}}$ - $\overrightarrow{\text{Pro}}$ - $\overrightarrow{\text{Ala}}$ - $\overrightarrow{\text{Asn}}$ - $\overrightarrow{\text{Phe}}$ - Arg	9

Determination by Edman degradation: arrow above the sequence, degradation performed directly on tryptic units; arrow under the sequence, degradation performed on chymotryptic fragments of tryptic unit. T_5Ch_1: chymotryptic fragment of peptide T_5 etc...

Table 2
Amino acid sequence of the middle part of the β chain.

35 40 45 50
Tyr - Phe - Thr - Thr - Phe - Gly - Asn - Leu - Gly - Ser - Ala - Asp - Ala - Ile - Cys - His -
←———— T_5 ————

55 60 65
- Asn - Ala - Lys - Val - Leu - Ala - His - Gly - Glu - Lys - Val - Leu - Ala - Ala - Ile - Gly -
————→←———— T_6 ————→←———— T_7 ————
←———— $TF_{III}Ch_1$ ————→←———— $TF_{III}Ch_2$ ————→←————

70 75 80
- Glu - Gly - Leu - Lys - His - Pro - Glu - Asn - Leu - Lys - Ala - His - Tyr - Ala - Lys - Leu -
————→←———— T_8 ————→←———— T_9 ————→←——
———— $TF_{III}Ch_3$ ————→←———— $TF_{III}Ch_4$ ————→←———— $TF_{III}Ch_5$ —

85 90 95 98
- Ser - Glu - Tyr - His - Ser - Asn - Lys - Leu - His - Val - Asp - Pro - Ala - Asn - Phe - Arg
———— T_{10} ————→←———— T_{11} ————→
————→←———— $TF_{III}Ch_6$ ————→

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