THE β-CHAIN OF FROG HEMOGLOBIN (*RANA ESCULENTA*). A 34 RESIDUE *N*-TERMINAL SEQUENCE

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The major hemoglobin of frog *Rana esculenta* has been purified by column chromatography on carboxymethyl-Sephadex and two types of chain, α and β, have been separated by counter-current distribution [1]. Determination of amino ends shows that the α-chain has an *N*-terminal sequence of Ac–Ala–Leu and the β-chain has an *N*-terminal glycine residue [2]. This paper described the determination of a 34 residue *N*-terminal sequence of the β-chain.

The β-chain of the reduced globin was isolated as previously described [1]. Cysteine residues were carboxymethylated according to Crestfield et al. [3] and lysine residues were trifluoroacetylated according to Goldberger and Anfinsen [4]. The derivative was subjected to tryptic hydrolysis (pH 8.0, 3 hr, 37°) with an enzyme/substrate weight ratio of 1:100. Because the β-chain has 4 arginine residues, 5 fragments TF_I, TF_{II}, TF_{III}, TF_{IV} and TF_V were present in the digest. The trifluoroacetyl groups were removed from the peptides by exposure to 1.0 M piperidine (2 hr, 5°). The resulting mixture of peptide fragments gave four separate fractions by gel-filtration on Sephadex G-50 in 0.1 M acetic acid. The components were purified by paper electrophoresis (pyridine-acetate buffer, pH 3.7, 40 V/cm, 80 min).

Table 1
Determination of the *N*-terminal sequence of the β-chain.

Peptide		Sequence	Number of residues
	T_1	1 2 3 4 5 6 7 8 9 10 11 Gly–Ser–Asp–Leu–Val–Ser–Gly–Phe–Trp——Gly–Lys ←——T_1Ch_1——×T_1Ch_2→←T_1Ch_4→ ←——T_1Ch_3——→	11
TF_I			
	T_2	12 13 14 15 16 Val–Asp–Ala–His–Lys	5
	T_3	17 18 19 20 21 22 23 24 Ile–Gly–Gly–Glu–Ala–Leu——Ala–Arg ←——T_3Ch_1——→ ←T_3Ch_2→	8
TF_{II}	T_4	25 26 27 28 29 30 31 32 33 34 Leu–Leu–Val–Val–Tyr–Pro–Trp–Thr–Gln–Arg ←T_4Ch_1→←——T_4Ch_2——×——T_4Ch_3→	10

→Determination by Edman degradation [7]. T_1Ch_1: chymotryptic fragment of peptide T_1, etc.

Of 5 fragments, only one has an *N*-terminal glycine residue; it is thus the *N*-terminal peptide, TF_1, of the β-chain. Its amino acid composition was determined according to Spackman et al. [5]. It has 24 residues two of which are lysine and one arginine. It was cleaved by trypsin into 3 peptides, T_1, T_2, T_3, which were separated by paper chromatoelectrophoresis [6]. The *N*-terminal residues are respectively Gly, Val and Ile and, because T_3 has a *C*-terminal residue of arginine, the order of the 3 peptides of fragment TF_I could be deduced. Amino acid compositions were determined and the sequences established by Edman degradation [7]. For T_1 and T_3, chymotryptic hydrolysis (0.1 M ammonium bicarbonate pH 8.0, 3 hr, 37°) with a enzyme/substrate weight ratio of 2:100, was used to complete or confirm the structure. Table 1 indicates the results.

The fragment TF_{II} which follows TF_I in the trifluoroacetylated β-chain was recognized by its *N*-terminal sequence, Leu–Leu, and by identification of a tetrapeptide Ala–Agr–Leu–Leu in chymotryptic digest of the β-chain. Because TF_{II} had only one basic residue, namely an arginine residue, it could also be isolated directly from a tryptic digest of β-chain. This peptide was called T_4 because it is the fourth tryptic unit of β-chain. It has 10 amino acid residues and the determination of its sequence is shown in table 1.

Because there is a strong homology between the sequence 23–40 of human β-chain and the sequence 17–34 of frog β-chain, it is supposed that a deletion has occurred in the *N*-terminal part of the frog β-chain. Such a deletion has already been observed for sheep hemoglobin C which lacks 4 residues in its *N*-terminal portion [8].

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