THE CHICKEN MYOGLOBIN (*GALLUS gallus*). A 47 RESIDUE N-TERMINAL SEQUENCE

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Received 24 March 1972

1. Introduction

Amino acid sequences have been determined on myoglobins only for land and marine mammals; therefore we have extended the comparative structural study to avian proteins.

The partial primary structure of myoglobin extracted from chicken heart has been established with sequence data obtained on peptides isolated from tryptic and chymotryptic hydrolysates. Our results have been confirmed by stepwise degradation of the whole globin using a sequencer.

From the overlaps between tryptic and chymotryptic peptides 44 of the first 47 residues of the protein chain have been positioned.

With the confirmation obtained by the use of a sequencer, this paper presents the 47-residue N-terminal fragment of chicken myoglobin.

2. Material and methods

The main component of chicken myoglobin was prepared as previously described [1].

After removing the heme moiety from the protein, the globin denatured by heat (10 min, 90°) was submitted to enzymatic digestion.

After addition of enzyme to the substrate solution (1.75: 100, w/w), chymotryptic digestion proceeded at pH 8.2 and 37° for 6 hr. The globin was subjected to tryptic hydrolysis (pH 8.0, 37°, 5 hr) with an enzyme/substrate weight ratio of 3:100.

The separation of chymotryptic and tryptic peptides was performed by column chromatography on Dowex 50 × 8 resin as described by Bradshaw and Gurd [2].

Each fraction was purified by high voltage electrophoresis on paper at pH 3.5 or 6.5 or by chromatography on Aminex A 5 using a double linear gradient of pyridine acetate (pH 3.1 to 5.0 and 0.2 to 2 N in pyridine).

Amino acid analysis of the peptides was done with an automatic amino acid analyzer (Beckman Unichrom) and peptide sequences were determined by the combined dansyl-Edman technique [3,4]. Dansyl amino acids were identified by two dimensional chromatography on polyamide sheets of 5.0 × 5.0 cm using solvents reported by Woods and Wang [5].

The C-terminal residues were identified by amino acid analysis after kinetic hydrolysis with carboxypeptidases A or B.

The whole globin was submitted to automatic Edman degradation [6] using the Beckman sequencer (model 890).

The identification of the PTH amino acids was obtained by gas chromatography [7] (Beckman GC 45 chromatograph) and in some cases was confirmed by analysis of the amino acid after acid hydrolysis of the derivative [8].

3. Results and discussion

The N-terminal sequence of chicken myoglobin

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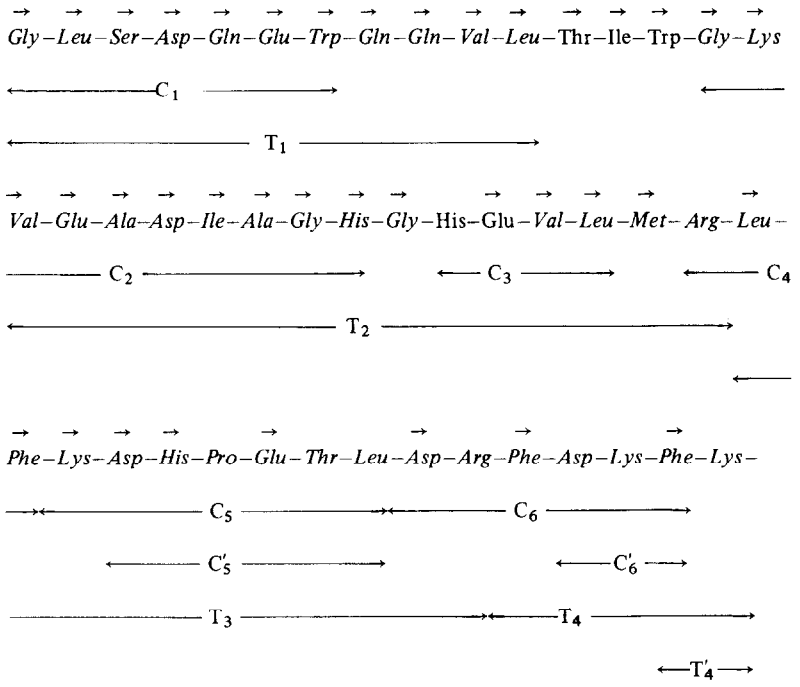
Table 1
Determination of the N-terminal sequence of chicken myoglobin.



←C or T→: chymotryptic or tryptic peptides. →: Amino acids identified without any ambiguity by automatic sequential degradation. Residues identified as dansyl derivatives or by carboxypeptidases are set in italic print.

obtained by chymotryptic and tryptic peptide overlaps and by automatic sequential degradation is presented in table 1.

The partial amino acid sequence of the chicken globin differs from that of the myoglobins of all other species already studied at 7 sites on the first 47 residues.

These replacements are a Gln for Gly at position 5, a Thr for His or Asn at position 12, a His for Glu, Gln or Lys at position 26, a Met for Ile at position 30, an Asp for Gly, Ser or Thr at position 35, an Asp for Glu at position 41 and an Arg for Lys at position 42. Preliminary physico-chemical studies in solution seem to indicate that the three dimensional structure of chicken myoglobin appears very similar to that of sperm whale myoglobin [9].

The partial primary structure reported in this paper thus extends the comparative studies of myoglobins to another zoological class: the birds.

As sequence studies undertaken in parallel on penguin (*Aptenodytes forsteri*) myoglobin are progressing well, we will be able in the very near future to make broader phylogenetic comparison of myoglobins at the amino acid sequence level.

Acknowledgements

Support from the Fonds National de la Recherche Scientifique and from the Fonds de la Recherche Fondamentale Collective is gratefully acknowledged. This work has also been supported by a grant from the National Science Foundation (GB 18483). We are grateful to Mr. Jacques Gallis for performing the amino acid analyses.

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