

PENGUIN HEMOGLOBIN (*APTENODYTES FORSTERI*). A 45 RESIDUE N-TERMINAL SEQUENCE

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1. Introduction

The hemoglobins have been studied from the point of view of their amino acid sequence principally in mammals; this study has also been extended to the fish (carp, lamprey) and to the invertebrates (*Chironomus thummi*, *Glycera dibranchiata*). Nevertheless, as far as birds are concerned, only the primary structure of the α -chain of the chicken major hemoglobin component is known at the present time. With the goal of expanding the bulk of knowledge with respect to structural comparative studies, the determination of the amino acid sequence of the α -chain of the penguin hemoglobin was undertaken.

The positions of the first 45 residues of this α -chain were determined by two different approaches: on the one hand from information obtained on the tryptic peptides of the two cyanogen bromide fragments of the α -chain, and on the other hand from results of the degradation of the entire polypeptide chain by use of the sequencer.

2. Materials and methods

The hemoglobin was extracted from erythrocytes by hemolysis as was previously described [1]. After extraction of the heme by HCl acidified acetone [2], the globin was successively submitted to reduction by mercaptoethanol and to aminoethylation of the

thiol groups by use of ethyleneimine [3]. The α - and β -chains were isolated from the aminoethylated globin [4].

The α -chain dissolved as per 1 mg per ml of 70% formic acid, was hydrolyzed by cyanogen bromide in excess as 100 moles per mole of protein. The fragments obtained by cleavage were fractionated by filtration on Sephadex G-25 fine column in 0.2 M acetic acid.

For the separated fragments the N-terminal residues were determined as dansylamino acids [5], whereas the C-terminal residues were identified by amino acid analysis after carboxypeptidases A or B hydrolysis.

The isolated fragments were digested by trypsin (pH 8.0, 37°C, 4 hr) with an enzyme/substrate weight ratio of 1:50. The separation of tryptic peptides was realized either by high voltage paper electrophoresis at pH 3.7 [6], or by Dowex 50 X-2 chromatography [7].

The amino acid analysis of the fragments obtained after use of cyanogen bromide and that of the tryptic peptides was realized with an automatic amino acid analyser (Beckman Unichrom).

The sequence of the peptides was determined by the dansyl-Edman technique [5, 8]. The chromatography methods of Woods and Wang [9] permitted the identification of dansyl amino acids.

The α -chain of the aminoethylated globin was submitted to Edman automatic degradation [10] with a Beckman sequencer (model 890). The PTH amino acids were identified by gas chromatography (Beckman GC 45 chromatograph) [11] or by thin-layer chromatography [12].

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and 14, of Ile and Phe whereas for the other known α -chains one finds Ala and Tyr.

In the 45 positioned residues, one finds in position 43 a phenylalanine which is invariant for all different known hemoglobins and myoglobins.

A more complete study of the total sequence of the α -chain of the penguin hemoglobin will permit more precise phylogenetic conclusions.

References

- [1] Schnek, A.G., Paul, C. and Leonis, J. (1966) International Symposium on comparative Haemoglobin Structure, Thessaloniki, p. 103.
- [2] Rossi-Fanelli, A., Antonini, E. and Caputo, A. (1968) *Biochim. Biophys. Acta* 30, 608.
- [3] Crestfield, A.M., Moore, S. and Stein, W.H. (1963) *J. Biol. Chem.* 288, 622.
- [4] Paul, C., Monier, C., Schnek, A.G., Brygier, J. and Leonis, J. (1968) *Arch. Internatl. Physiol. Biochem.* 76, 383.
- [5] Gray, W.R. and Hartley, B.S. (1963) *Biochem. J.* 89, 59.
- [6] Katz, A.M., Dreyer, W.S. and Anfinsen, C.B. (1959) *J. Biol. Chem.* 234, 2897.
- [7] Schroeder, W.A., Jones, R.T., Cornick, J. and McCalla, K. (1962) *Anal. Biochem.* 34, 1570.
- [8] Dopheide, T.A.A., Moore, S. and Stein, W.H. (1967) *J. Biol. Chem.* 242, 1833.
- [9] Woods, K.R. and Wang, K.T. (1967) *Biochim. Biophys. Acta* 133, 369.
- [10] Edman, P. and Begg, C. (1967) *European J. Biochem.* 1, 80.
- [11] Pisano, J.J. and Bronzert, T.J. (1969) *J. Biol. Chem.* 244, 5597.
- [12] Edman, P. (1970) in: *Protein Sequence Determination*, (Needleman, S.B., ed.), p. 211, Springer-Verlag, Berlin, Heidelberg, New York.
- [13] Matsuda, G., Takei, H., Ku Chayu, Wu and Shiozowa, T. (1971) *Internatl. J. Protein Res.* 3, 173.
- [14] Braunitzer, G., Gehring-Muller, R., Hilschmann, N., Hilse, K., Hobam, G., Rudloff, V. and Wittman-Liebold, B. (1961) *Hoppe Seyler's Z. Physiol. Chem.* 325, 283.
- [15] Hill, R.J., Konigsberg, W., Guidotti, G. and Craig, L.C. (1962) *J. Biol. Chem.* 237, 1549.