THE PRIMARY STRUCTURE OF THE MONOMERIC HEMOGLOBIN (ERYTHROCRUORIN) COMPONENT CTT-I OF CHIRONOMUS THUMMI THUMMI (INSECTA, DIPTERA)

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The amino acid sequence of the monomeric component CTT-I, one of 12 of the hemoglobins (Erythrocruorins) of larvae of Chironomus thummi thummi (Insecta, Diptera) is given. The sequence and the side chains forming the heme complex are discussed.

1. Introduction

In Chironomus thummi thummi larvae (Insecta, Diptera) at least twelve electrophoretically different hemoglobins are present[1,2]. At physiological pH, some of these hemoglobins are monomeric and some are dimeric[2]. This high degree of polymorphism has attracted our interest in the structural, functional as well as the genetic aspects of these hemoglobins. The primary structure of one of the monomeric

and three of the dimeric hemoglobins have already been reported[3,4,5,6]. In this paper, we report the primary structure of one of the monomeric components, CTT-I.

2. Materials and methods

2.1 Isolation of hemoglobin

Hemoglobin was isolated from the larvae according to the method already described[2]. CTT-I, the most basic of chironomus hemoglobins, was isolated by DEAE-Cellulose column chromatography. Homogeneity of the component was checked by polyacrylamide gel electrophoresis.

2.2 Enzymatic digestion and separation of peptides

Tryptic peptides were obtained by digestion with trypsin (TPCK-treated). Peptic peptides were obtained by digestion with pepsin for 2 hours in 0.1 N HCl. Small peptides were first separated by gel filtration on Sephadex G-25 in 0.1 M acetic acid. The different peaks were subsequently chromatographed on Dowex 1X2 or Dowex 50X4. Larger peptides were purified on DEAE-Cellulose with buffers containing 8 M urea.

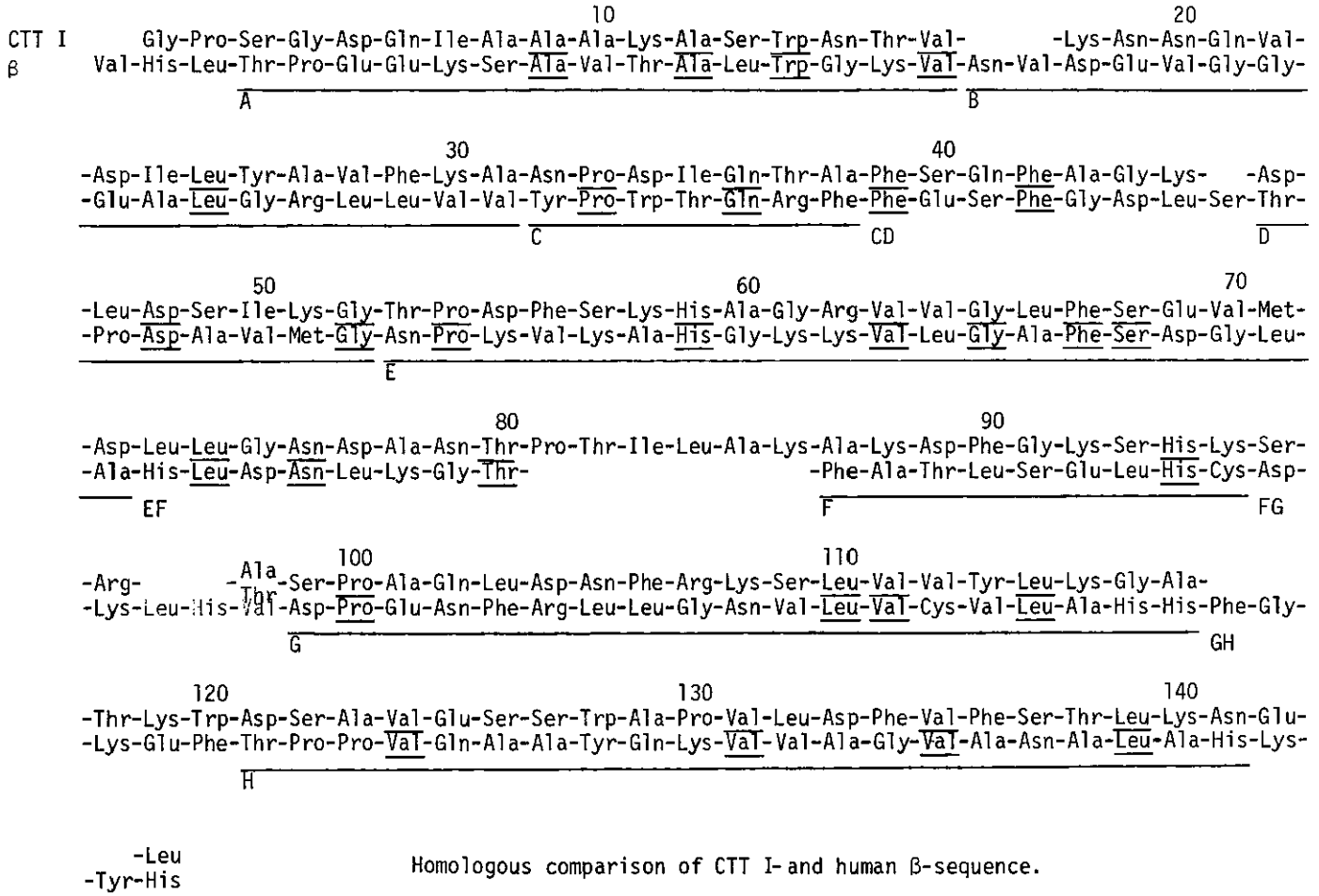
2.3 Sequence determination

Sequence determination was done by automatic Edman degradation[7] in Beckman Sequencer. Two programmes were applied, a) Quadrol Programme and b) N,N-Dimethylaminopropyne Programme[8]. Quadrol Programme was applied for sequencing intact polypeptide chain, large peptides and some lysyl peptides which had been reacted with reagent IV (1,3,5-trisulfonic-7-isothiocyanatonaphthalene, trisodium salt[9])

N,N-Dimethylaminopropyne Programme was applied for small lysyl peptides and arginyl peptides. The PTH-amino acids were identified by thin-layer chromatography and by high performance liquid chromatography.

3. Discussion

To determine the primary structure of CTT-I the peptides were isolated and sequenced after digestion with trypsin. The alignment of the peptides was achieved by means of peptic peptides. The peptide chain of CTT-I is comprised of 143 amino acids. Position 98 is occupied by the amino acids alanin and threonin in the ratio 1:1. In the figure CTT-I is compared homologously with the ß-chains of human hemoglobin. CTT-I is longer by 6 amino acids than the monomeric CTT-III, but shorter than the dimeric components of Chironomus hemoglobins, sequenced so far. The physico-chemical properties, investigated by Gersonde[10,11], show a higher similarity with myoglobin than with hemoglobin. In comparison with the human α- and ß-chains one can find 27 and 29 identical amino acids respectively, in comparison with sperm-whale myoglobin 23 identical amino acids are found. CTT-III and CTT-I have 62 amino acids in identical positions. The homology is relatively small. The distance between the distal and the proximal histidin (E7, F8) of CTT-I is 34 amino acids, that is 6 more than in the case of the vertebrate α- and ß-chains, the myoglobins and CTT-III. It is suggested, that this increase of the chain length in the



Homologous comparison of CTT I- and human β-sequence.

EF-region leads to a larger heme pocket, as it is described for leg hemoglobin (lupinus luteus)[12] and is also postulated for the dimeric hemoglobins CTT-IIß and CTT-VIIB. Other differences in the chainlength are likely to be of little importance for the tertiary structure. In the AB-corner CTT-I has a deletion of two amino acids compared with the ß-chains and of four amino acids compared with the α-chains, which is also found with CTT-III. A D-Helix probably exists. Differing from the other CTT-hemoglobins there are two deletions in the FG-region compared with the human chains. In the GH-region exist two deletions, in the HC-region one deletion is found. CTT-I is the only component of the CTT-hemoglobins, sequenced so far that has a valin in E11 as most of the known hemoglobins and myoglobins. In all the other CTT-hemoglobins this functionally important side chain is isoleucin[13]. The distal and the proximal histidin in E7 and F8 are present. The hydrophobic amino acids of the heme pocket were found in CTT-I in the corresponding positions.

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References

1 G. Braunitzer and V. Braun, Hoppe-Seyler's Z. Physiol. Chem. , 1965, 340, 88-91.

2 V. Braun, R.R. Crichton and G. Braunitzer, Hoppe-Seyler's Z. Physiol. Chem., 1968, 349, 197-210.

3 G. Buse, S. Braig and G. Braunitzer, Hoppe-Seyler's Z. Physiol. Chem., 1969, 350, 1686-1690.

4 T. Kleinschmidt and G. Braunitzer, Hoppe-Seyler's Z. Physiol. Chem., 1976, 357, 1805-1808.

5 D. Sladić-Simić, T. Kleinschmidt and G. Braunitzer, Hoppe-Seyler's Z. Physiol. Chem., 1977, 358, 591-594.

6 R. Lalthantluanga and G. Braunitzer, Hoppe-Seyler's Z. Physiol. Chem., in press.

7 P. Edman and G. Begg, Eur. J. Biochem., 1967, 1, 80-91.

8 G. Braunitzer and B. Schrank, Hoppe-Seyler's Z. Physiol. Chem., 1970, 351, 417.

9 G. Braunitzer, B. Schrank, A. Ruhfus, S. Petersen and U. Petersen, Hoppe-Seyler's Z. Physiol. Chem., 1971,352, 1730-1732.

10 A. Wollmer, G. Buse, H. Sick and K. Gersonde, Eur. J. Biochem., 1972, 24, 547-552.

11 K. Gersonde, H. Sick, A. Wollmer and G. Buse, Eur. J. Biochem., 1972, 25, 181-189.

12 B.K. Vainstein, E.H.Arutyunyan, I.P. Kuranova, V.V. Borisov, N.I. Sosfenov, A.G. Pavlovskii, A.I.Gebrenko, N.V. Konareva and I.V. Nekrasov, Dokl. Akad. Nauk SSSR, Ser. Biokhim. 1977, 233, 238-241.

13 R. Huber, O. Epp and H. Formanek, J. Mol. Biol., 1969, 42, 591-594.

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