The Primary Structure of the Monomeric Hemoglobin (Erytrocruorin) Component CTT IV of *Chironomus thummi thummi* (Insecta, Diptera)

J. Pfletschinger, H. Plagens, and G. Braunitzer

Max-Planck-Institut für Biochemie, Abteilung Proteinchemie, D-8033 Martinsried

Z. Naturforsch. **35 c,** 840–843 (1980);
received May 23, 1980

Hemoglobin, *Chironomus*

The primary structure of the monomeric hemoglobin (Erythrocruorin) CTT IV from larvae of *Chironomus thummi thummi* (Diptera) is presented. The sequence was determined automatically. The primary structure is compared with human α-globin-chain.

In *Chironomus thummi thummi* larvae (Insecta, Diptera) at least twelve electrophoretically different hemoglobins are present [1–3]. At physiological pH, some of these hemoglobins are monomeric and some are dimeric [4]. 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 two of the monomeric and five of the dimeric homoglobins have already been reported [5–10]. The tertiary structure of CTT III is now examined in 1.4 Å resolution [11]. In this paper we report on the primary structure of one of the monomeric components, CTT IV.

Materials and Methods

Hemoglobin was isolated from the larvae according to the method already described [4]. The hemoglobins were separated in monomers and dimers by gel chromatography on Sephadex G-75 (column: ∅ = 10 cm, h = 90 cm, flow rate 300 cm³/h; buffer: 0.025 m potassium phosphate pH 6.0 with 0.5% thiodiglycoll). Hemoglobin of CTT IV was isolated by DEAE Sephadex A-25 column chromatography (column: ∅ = 2.5 cm, h = 10 cm; buffer: 0.1 tris-aminomethan-HCl, pH = 9.0; gradient: 0.1 M to 0.3 M NaCl; gradient volume: 600 cm³. Homogenity of the component was checked by polyacrylamide gel electrophoresis and by N-terminal degradation in the sequencer.

Digestions

a) Enzymatic digestion. The tryptic and the tryptic arginyl peptides were obtained by digestion with TPCK-reacted trypsin of native and of modified globin, respectively, by blocking ε-amino groups of lysine with malein anhydride [12].

b) Chemical digestion. The cleavages at Asp-Pro bonds with 80% formic acid/7 M guanidinium-chloride solution [13] and at methionine residues with cyanogen bromide [14] were also carried out to obtain some overlapping peptides.

Separation of peptides

The cyanogen bromide peptides were fractionated by gel filtration on Sephadex G-50 in 8 M urea (pH 3.5, with conc. HCl; column ∅ = 2.5 cm, h = 100 cm). The arginyl peptides were separated by gel filtration on Sephadex G-50 in 6 M guanidinium-chloride (pH 8.6, 0.1 M trisaminomethan-HCl; column ∅ = 2.5 cm, h = 100 cm).

Amino acid analysis

Samples were hydrolyzed in constantly boiling 5.7 N hydrochloric acid at 110 °C for 20 h. The analyses were performed with a Beckman Model 121 C amino acid analyzer.

Sequence determination

This was done by automatic Edman degradation in a Beckman Sequencer, Model 890 B or 890 C [15]. Two programmes were applied:

a) Quadrol programme [15]: This was applied for sequencing the intact polypeptide chain and large peptides. This programme has an extended solution phase in the quadrol buffer [16].

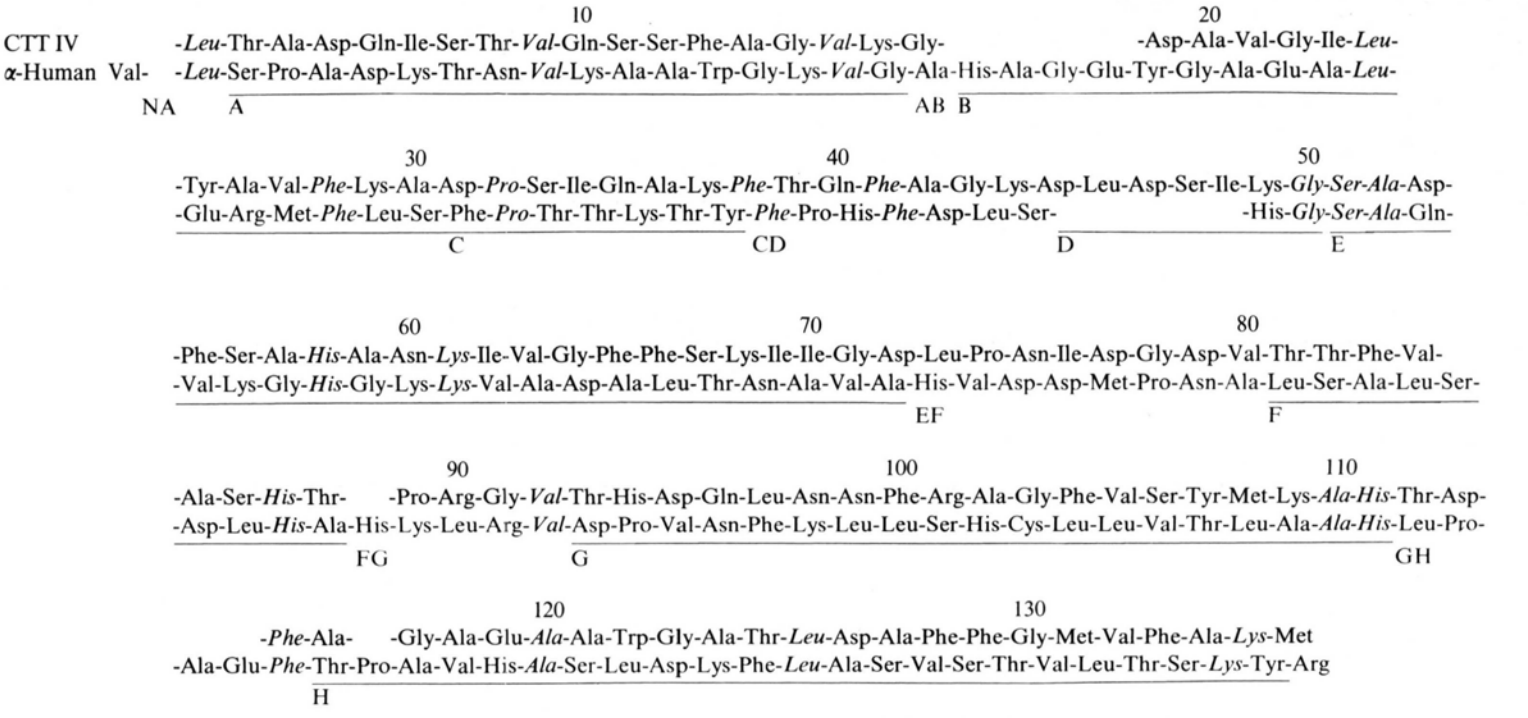
b) N,N-Diethylaminopropine programme [17]: This was applied for tryptic and modified [18] cyanogen bromide peptides.

Identification of phenylthiohydantoin derivatives of amino acids: The derivatives were identified by Thin-layer and by high performance liquid chromatography [19].

Abbreviations: CTT, *Chironomus thummi thummi;* CTT IV, Hemoglobin of *Chironomus thummi thummi,* component IV.

Reprint requests to Prof. Dr. G. Braunitzer.

0341-0382/80/0900-0840 $ 01.00/0



Homologous comparison of the human α-chain and the hemoglobin of CTT IV.

Results and Discussion

The primary structure of a monomeric hemoglobin, CTT IV, of *Chironomus thummi thummi* was determined by automatic Edman degradation of the intact polypeptide chain and its overlapping peptides.

CTT IV has 136 amino acid residues. The analysis of the protein is given in Table I. The hemoglobin CTT IV has 3 methionines, 1 tryptophane, 4 histidines and a large number of hydrophobic amino acids, but it lacks cysteine. In Fig. 1 CTT IV is compared for homology [20] with the α-chains of human hemoglobin. The indices of the helix are according to Perutz [21]. The underlined amino acids are identical. Only 21 positions are invariant, this accounts for 15%.

Table I. Amino acid composition of CTT IV. (20 h hydrolysis.)

	CTT IV	Sequenz
Asp	18.6	16
Thr	8.6	9
Ser	10.1	10
Glu	6.6	6
Pro	2.7	3
Gly	12.9	13
Ala	19.4	20
Val	10.1	10
Met	2.9	3
Ile	6.7	6
Leu	6.1	6
Tyr	1.6	2
Phe	14.8	14
Lys	9.5	9
His	3.7	4
Arg	2.1	2
Trp		1
		136

Some features of the sequence may be discussed. The chain starts with the non-helical region Leu NA3 (1). In the NA region the human α-chain is by one amino acid longer. The helix begins with Thr A1 (2) and ends in both chains with A16. The B helix of CTT IV is in the N-terminal region by one turn shorter [11] and extends from Asp B5 (19) to Ala B16 (30). The C helix follows from Asp C1 (31) to Lys C7 (37). The C helix and the CD region have the same number of amino acids in both chains. The D helix is not present in human α-hemoglobin chains.

The D helix in CTT IV begins from Asp D1 (45) to Gly D7 (51). The E-helix, the interhelical region and the F helix have the same number of residues in CTT IV and in the human α-chains. The FG segment Pro FG2 (89) to Val FG4 (93) in CTT IV has one amino acid less and extends to the G helix Thr G1 (94) to His G19 (111). The EH region in CTT IV is two residues shorter than the human α-chain. Presumably the H-helix goes from Ala H1 (115) to Met H23 (136). The H-helix in the α-chains has two amino acids more [20].

The comparison of the CTT hemoglobins shows identity of CTT IV with CTT III [5] in 83%, with CTT I [6] in 41% and with CTT IIβ [7] only in 34% of the residues. This polymorphism is also reflected in the different physico-chemical properties [22–26] of the individual components.

Acknowledgements

The excellent technical assistance of Miss B. Schrank, Mr. A. Stangl, Mr. C. Krombach, Miss U. Scheithauer, and Mrs. E. Hannes is gratefully acknowledged.

[1] G. Braunitzer and V. Braun, Hoppe-Seyler's Z. Physiol. Chem. **340,** 88 (1965).
[2] G. Braunitzer, H. Glossmann, and J. Horst, Hoppe-Seyler's Z. Physiol. Chem. **349,** 1789 (1968).
[3] H. Tichy, Chromosoma **29,** 131 (1970).
[4] V. Braun, R. R. Crichton, and G. Braunitzer, Hoppe-Seyler's Z. Physiol. Chem. **349,** 197 (1968).
[5] G. Buse, G. Steffens, G. Braunitzer, and W. Steer, Hoppe-Seyler's Z. Physiol. Chem. **360,** 89 (1979).
[6] T. Kleinschmidt, H. v. d. Mark-Neuwirth, and G. Braunitzer, Heterocycles **10,** 251 (1978).
[7] T. Kleinschmidt and G. Braunitzer, Liebigs Ann. Chem. **1978,** 1060.
[8] D. Sladic-Simic, T. Kleinschmidt, and G. Braunitzer, Hoppe-Seyler's Z. Physiol. Chem. **358,** 591 (1977).
[9] W. Steer and G. Braunitzer, in Vorbereitung.
[10] H. Aschauer, Z. H. Zaidi, and G. Braunitzer, Hoppe-Seyler's Z. Physiol. Chem. **360,** 1513 (1979).
[11] W. Steigemann and E. Weber, J. Mol. Biol. **127,** 309 (1979).
[12] P. J. G. Butler, I. J. Harris, B. S. Hartley, and R. Leberman, Biochem. J. **103,** 78 (1967).
[13] J. Jauregui-Adell and J. Marti, Analyt. Biochem. **69,** 468 (1975).
[14] E. Gross and B. Wittkop, J. Am. Chem. Soc. **1961,** 1510.
[15] P. Edman and G. Begg, Eur. J. Biochem. **1,** 80 (1967).
[16] J. Thomsen, T. Bucher, K. Brunfeldt, E. Nexö, and H. Olesen, Eur. J. Biochem. **69,** 87 (1976).
[17] G. Braunitzer and B. Schrank, Hoppe-Seyler's Z. Physiol. Chem. **351,** 417 (1970).
[18] G. Braunitzer and J. Pfletschinger, Hoppe-Seyler's Z. Physiol. Chem. **359,** 1015 (1978).
[19] C. L. Zimmerman and J. J. Pisano, Methods in Enzymology, **Vol. XLVII,** p. 45, (C. H. W. Hirs and S. N. Timasheff, eds.).
[20] G. Braunitzer, R. Gehring-Müller, N. Hilschmann, K. Hilse, G. Hobom, V. Rudloff, and B. Wittmann-Liebold, Hoppe-Seyler's Z. Physiol. Chem. **325,** 283 (1961).
[21] M. F. Perutz, J. Mol. Biol. **13,** 646 (1965).
[22] A. Wollmer, G. Buse, H. Sick, and K. Gersonde, Eur. J. Biochem. **24,** 547 (1972).
[23] K. Gersonde, H. Sick, A. Wollmer, and G. Buse, Eur. J. Biochem. **25,** 181 (1972).
[24] G. Steffens, G. Buse, and A. Wollmer, Eur. J. Biochem. **72,** 201 (1977).
[25] A. Wollmer, G. Steffens, and G. Buse, Eur. J. Biochem. **72,** 207 (1977).
[26] G. N. La Mar, M. Overkamp, H. Sick, and K. Gersonde, Biochemistry **17,** 352 (1978).