CHICKEN ERYTHROCYTE HISTONE H_5
III. SEQUENCE OF THE AMINO-TERMINAL HALF OF THE MOLECULE (III RESIDUES)

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1. Introduction

In our previous papers [1,2] we have presented the N-terminal sequence (70 residues) of the chicken erythrocyte histone H_5^*, and a sequence of 25 residues around the only phenylalanyl residue present in that protein. The amino acid sequence of the histone H_5 has now been extended up to the 111th residue. The sequence was determined from data provided by the peptides obtained by hydrolysis of the protein or of its carboxy-terminal fragment NB-4, with trypsin, thermolysin and dilute acetic acid.

2. Materials and methods

All materials and methods were essentially as described previously [1,2] with the following additions. The histone H_5 was hydrolyzed at 105°C for 6 h with 0.25 M acetic acid (10 mg protein/ml acetic acid). The hydrolysate was fractionated on Sephadex G-50 F equilibrated and eluted with 0.01 N HCl. On the other hand the histone H_5 was hydrolyzed at 37°C for 4 h in 0.1 M ammonium bicarbonate pH 8.0 with trypsin at an enzyme/substrate ratio of 1:100. The hydrolysate was fractionated on Chromobeads P column (Technicon) with pyridine formate and pyridine acetate buffers [3]. Furthermore, the C-terminal fragment NB-4 (142 residues) obtained by NBS** cleavage of the protein [1] was hydrolyzed at 40°C for 2 h in 0.1 M ammonium bicarbonate pH 8.0, with thermolysin at an enzyme/substrate ratio of 1:100. The hydrolysate was fractionated on Sephadex G-25 F equilibrated and eluted with 0.01 N HCl. The fractions containing the small peptides ranging from di- to penta-peptides were further fractionated by ion-exchange chromatography, as described above.

3. Results and discussion

Among the peptides obtained by acetic acid cleavage of two aspartyl bonds in the histone H_5, one is of peculiar interest for the purpose of the present paper.

This peptide designated by Ac-2 contains 34

*The new histone nomenclature used here was accepted by the participants at the CIBA Foundation Symposium on the Structure and Function of Chromatin, April 3–5, 1974. This new nomenclature which has been proposed to the appropriate international nomenclature committee is as follows for each histone where the previous names are given in brackets: H_1 (F_1, I, KAP); H_2A (F_{2a2}, II_{b1}, ALK); H_2B (F_{2b}, II_{b2}, KSA); H_3 (F_3, III, ARK); H_4 (F_{2a1}, IV, GRK) and H_5 (F_{2C}, V, KAS).

**Abbreviation:* NBS, *N*-bromosuccinimide.

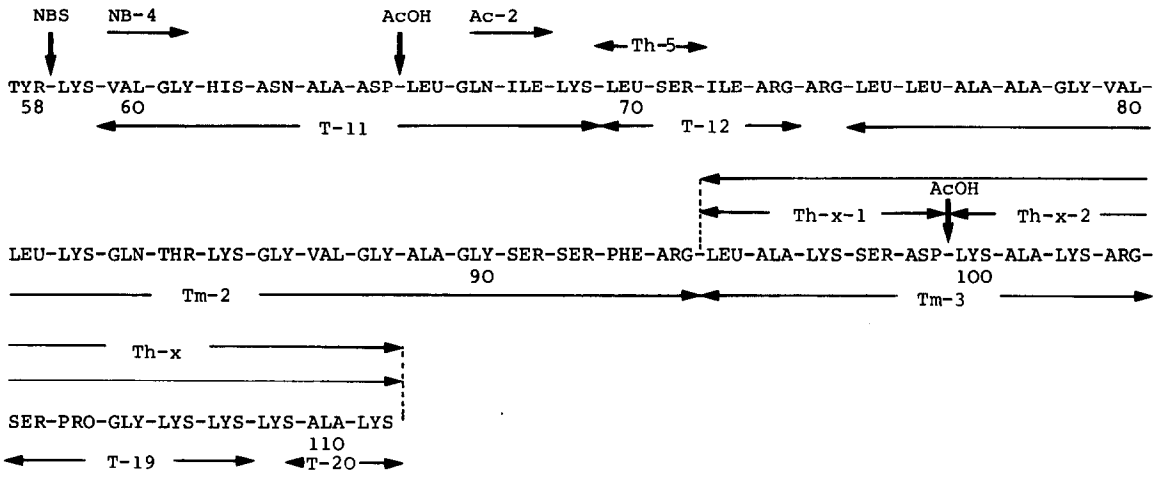

Fig.1. Ordering of the peptides from residue 58 to residue 111 in the amino acid sequence of chicken erythrocyte histone H_5. Tryptic peptides are designated by T- and Tm- (from maleylated peptide NB-4), and thermolytic peptides by Th-. Sites of cleavage of the protein by *N*-bromosuccinimide and acetic acid are indicated by NBS and AcOH respectively.

residues determined as follows: Asp, Thr, Ser_4, Glu_2, Gly_4, Ala_4, Val_2, Ile_2, Leu_6, Phe, Lys_4, Arg_3. The presence of aspartic acid in this peptide is related to an incomplete enucleation of the aspartyl residue due to a short time hydrolysis with dilute acetic acid. The amino-terminal sequence of peptide Ac-2 was found to be: Leu–Gln–Ile–Lys–Leu. This sequence corresponds to the carboxy-terminal sequence of the 70 first residues of the protein [1].

Its carboxy-terminal sequence Leu–Ala–Lys–Ser–Asp was deduced from the kinetic study of the hydrolysis of the peptide with carboxypeptidase C.

These structural studies as well as the amino acid composition of the peptide which contains the only

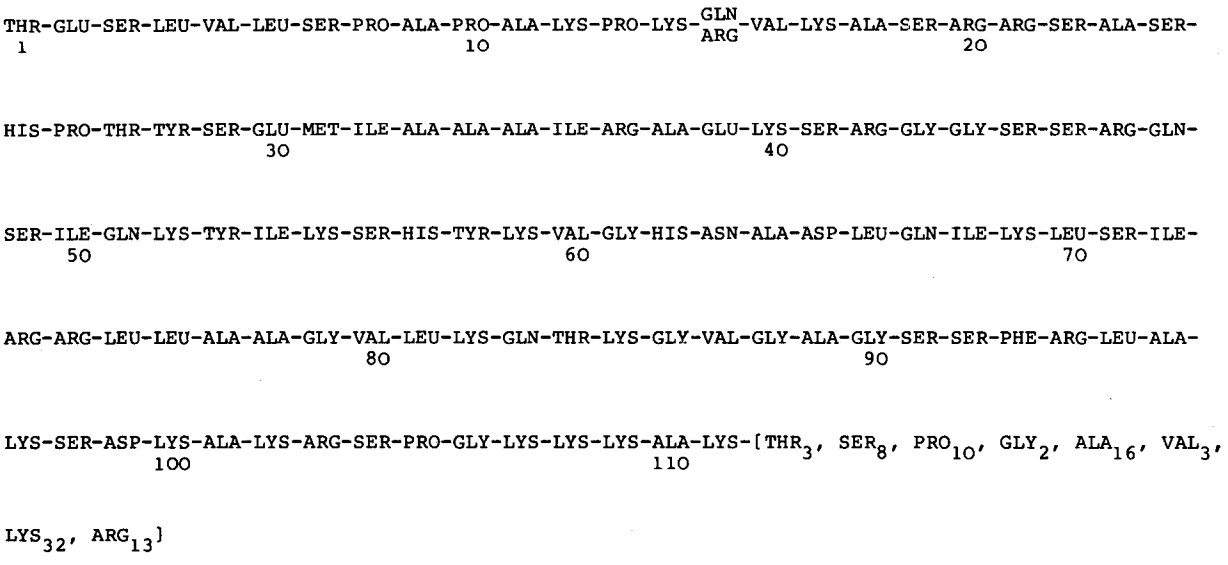

Fig.2. Sequence of the amino-terminal half of the chicken erythrocyte histone H_5.

phenylalanyl residue present in the histone H_5 indicate obviously that the sequence adjacent to the phenylalanyl residue established previously [2] comes next to the leucyl residue in position 70 (fig.1).

The identification of peptide T-12 Leu–Ser–Ile–Arg in the tryptic hydrolysate of the protein and of peptide Th-5 Leu–Ser in the thermolysin hydrolysate of the peptide NB-4 confirm the above assignment.

From the same thermolysin hydrolysate a highly basic peptide was isolated. This peptide Th-x (17 residues) has the following composition: Asp, Ser_2, Pro, Gly, Ala_3, Leu, Lys_7, Arg.

The peptide Th-x has a leucyl residue in the amino-terminal position. Its carboxy-terminal sequence Lys–Lys–Lys–Ala–Lys was established from the kinetic study of the hydrolysis of the peptide with carboxypeptidases B and A.

Its complete sequence was deduced from the structural data provided by the derived peptides Th-x-Ac-1 and Th-x-Ac-2 obtained by cleavage of the peptide Th-x with 0.25 M acetic acid and by the tryptic peptides Tm-3 and T-19 (fig.1). The peptide Tm-3 was identified in the tryptic hydrolysate of the maleylated peptide NB-4 whereas the peptide T-19 was isolated from the tryptic hydrolysate of the protein.

The amino-terminal sequence of the peptide Th-x overlaps the carboxy-terminal sequence of the peptide Ac-2 (fig.1).

These results led us to present the sequence of the first 111 amino acids of the chicken erythrocyte histone H_5 (fig.2). This sequence which corresponds to about half of the protein molecule, contains all the aromatic residues and most of the hydrophobic residues but alanine present in the histone H_5.

On the other hand, the highly basic character of the carboxy-terminal part of the protein appears in the composition (in brackets, fig.2) where half of the amino acids which remain to be sequenced, are basic.

This strongly suggests that all the globular structure of the whole molecule is located in the sequence 1–99.

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References

[1] Garel, A., Mazen, A., Champagne, M., Sautiere, P., Kmiecik, D., Loy, O. and Biserte, G. (1975) FEBS Lett. 50, 195–199.

[2] Sautiere, P., Kmiecik, D., Loy, O., Briand, G., Biserte, G., Garel, A. and Champagne, M. (1975) FEBS Lett. 50, 200–203.

[3] Sautiere, P., Moschetto, Y., Dautrevaux, M. and Biserte, G. (1970) Eur. J. Biochem. 12, 222–226.