CHICKEN ERYTHROCYTE HISTONE H_5; II AMINO ACID SEQUENCE ADJACENT TO THE PHENYLALANINE RESIDUE

P. SAUTIERE, D. KMIECIK, O. LOY, G. BRIAND and G. BISERTE
Unité 124 de l'Institut National de la Santé et de la Recherche Médicale, Boîte postale 3567, 59020-Lille CEDEX, France

and

A. GAREL and M. CHAMPAGNE
Institut de Biologie Moléculaire et Cellulaire, 15, rue Descartes, 67000-Strasbourg, France

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1. Introduction

In the first paper of this series we have presented the N-terminal sequence (70 residues) of the chicken erythrocyte histone H_5* [1]. We report here the sequence of 25 residues around the only phenylalanyl residue present in the histone H_5. This region rich in hydrophobic residues, shows a strong analogy with the sequence adjacent to the phenylalanyl residue 106 in the Rabbit thymus lysine-rich histone (RTL-3) [2]. The sequence data were obtained from a tryptic peptide of the maleylated protein, and from peptides obtained by hydrolysis of the unmodified protein with chymotrypsin and a staphylococcal protease.

2. Materials and methods

All materials and methods were essentially as reported in the earlier paper with the following addition. The histone H_5 was hydrolyzed at 37°C for 18 hr in 0.05 M ammonium bicarbonate, pH 8.0, with a staphylococcal protease** at an enzyme/substrate ratio of 1:30.

The hydrolysate was fractionated on Sephadex G-50 F, equilibrated and eluted with 0.01 N HCl. Further purification of the peptides was achieved either by paper chromatography or paper electrophoresis or both methods as described previously [3].

3. Results and discussion

In the tryptic hydrolysate of the maleylated histone a peptide containing the only phenylalanyl residue present in the protein was identified. This peptide (20 residues) has the following amino acid composition: Thr, Ser_2, Glu, Ala_3, Val_2, Leu_3, Phe, Lys_2, Arg. The sequence of the first sixteen residues was established by the automated Edman degradation (table 1).

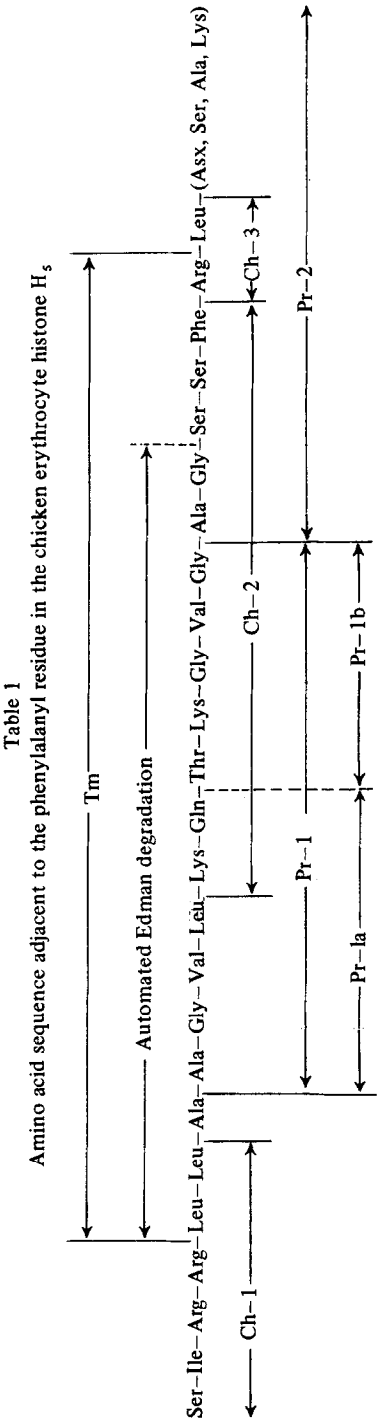
The remainder of the sequence was deduced from the data provided by the peptide Pr–2 isolated from the staphylococcal hydrolysate of the protein. This peptide has the following composition: Asp, Ser_3, Gly, Ala_2, Leu, Phe, Lys, Arg. The sequence of the first seven residues was as follows:

Ala–Gly–Ser–Ser–Phe–Arg–Leu–(Asx, Ser, Ala, Lys)

* 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_{2a_2}, II_{b_1}, ALK); H_2B (F_{2b}, II_{b_2}, KSA); H_3 (F_3, III, ARK), H_4 (F_{2a_1}, IV, GRK) and H_5 (F_{2C}, V, KAS).

** The staphylococcal protease isolated from *Staphylococcus aureus* strain V8 was a generous gift from Dr G. R. Drapeau.

Table 1
Amino acid sequence adjacent to the phenylalanyl residue in the chicken erythrocyte histone H_5



Due to a low recovery of this peptide no further sequence work could be carried out. However, this peptide allows us to localise the last of the three residues of aspartic acid present in the histone H_5 close to the phenylalanyl residue.

From the same hydrolysate we have isolated the peptide Pr–1 and its two derived peptides Pr–1a and Pr–1b. The peptide Pr–1 overlaps the median part of the tryptic peptide (table 1). The presence of the peptides Pr–1, Pr–1a, Pr–1b and Pr–2 in the staphylococcal protease hydrolysate led us to question the purity of the enzyme preparation.

Among the three proteases produced by the strain V8 of *Staphylococcus aureus* [4,5], one has been shown to have a specificity limited to glutamyl bonds in ammonium bicarbonate or ammonium acetate buffers [6,7].

The results that we have obtained clearly indicate that the 'Glu-specific protease' was contaminated by a protease which exhibits a thermolysin like activity, since bonds such as Ala–Ala, Gln–Thr, and Gly–Ala present in the peptide Tm were split.

Only three of the chymotryptic peptides called Ch–1, Ch–2, and Ch–3 are critical to the purposes of this report and will be described here.

The sequence of the peptide Ch–1 was found to be:

Ser–Ile–Arg–Arg–Leu–Leu

A derived peptide with only one leucine was also identified in the chymotryptic hydrolysate and its structure is:

Ser–Ile–Arg–Arg–Leu

Since we know from the amino acid analysis that the fragment NB–4 obtained by *N*-bromosuccinimide cleavage of the histone H_5, contains six leucine residues already positioned [1], it is obvious that the peptide Ch–1 overlaps the N-terminal part of the tryptic peptide Tm (table 1).

The peptides Ch–2, –Lys–Gln–Thr–Lys–Gly–Val–Gly–Ala–Gly–Ser–Ser–Phe, and Ch–3, –Arg–Leu–, overlap the C-terminal part of this peptide Tm (table 1).

Thus we have determined in the chicken erythrocyte histone H_5 a sequence of 25 residues, which includes the only phenylalanyl residue present in the histone molecule.

This sequence is in excellent agreement with the partial results obtained by Greenaway [8] on tryptic and thermolysin peptides of the histone H_5.

A striking analogy can be observed (table 2) by comparison of this sequence with the sequence adjacent to the phenylalanine residue 106 in the rabbit thymus/lysine-rich histone (RTL-3) determined by Jones et al. [2].

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Table 2
Sequence analogies in the vicinity of the phenylalanyl residue in histones H_1 and H_5

H_5 (Chicken erythrocyte)	Val–Leu–Lys–Gln–Thr–Lys–Gly–Val–Gly–Ala–Gly–Ser–Ser–Phe–Arg		
H_1 (Rabbit thymus) [2] RTL–3	Val– 96	–Glx–Thr–Lys–Gly–Thr–Gly–Ala–Ser–Gly–Ser–Phe–Lys 100	107

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