CHICKEN ERYTHROCYTE HISTONE H_5 ; I AMINO TERMINAL SEQUENCE (70 RESIDUES)

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1. Introduction

In addition to the five histones common to most vertebrate species, a specific histone (histone H_5)* occurs in the nucleated erythrocyte of bird, amphibian, reptile and fish. This histone has been isolated from chicken erythrocytes and described by Neelin et al. [1] and Hnilica [2].

Histone H_5 is thought to be synthesized in place of a certain amount of histone H_1 in mature chicken erythrocyte, since the two histones together equal the usual amount of histone H_1 present in the other tissues [3]. Although related to each other by their solubility in 5% perchloric acid or trichloracetic acid and by their molecular size, the histones H_1 and H_5 differ markedly by their amino-acid composition and particularly in their arginine content [4,5].

The amino acid sequence of the N-terminal fragment obtained by cyanogen bromide cleavage of the protein was determined by Greenaway and Murray [6]. Moreover, structural studies on the tryptic and thermolytic peptides of histone H_5 were reported by Greenaway [7].

The amino-terminal sequence of the histone H_5 has now been extended up to the 70th residue. This sequence was determined from data provided by tryptic and chymotryptic peptides, by NBS** fragments, and by the automated Edman degradation of the C-terminal fragment obtained by cyanogen bromide cleavage of the histone.

2. Materials and methods

Histone H_5 was prepared as described previously [4,5]. The purity of the protein was assessed by electrophoresis in polyacrylamide gel according to Leboy [8]. The amino acid composition was established on 24 hr and 72 hr hydrolysates.

Cyanogen bromide cleavage and the separation of the fragments of the protein were performed as reported previously [9]. A further purification of the C-terminal fragment CN–2 was achieved by preparative electrophoresis in vertical slab gels using the same conditions as for disc electrophoresis. The automated Edman degradation of this fragment was carried out in a SOCOSI model PS 100 Sequencer, with the Quadrol method.

NBS cleavage of histone H_5 was performed at room temperature, using 21 μmol of NBS per μmol of protein [10]. The reaction was monitored spectro-

* 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).

** *Abbreviations*: TPCK, L-(1-tosylamido-2-phenyl)-ethyl chloromethyl ketone. NBS, *N*-bromosuccinimide. PTH, Phenylthiohydantoin.

photometrically at 260 nm. The fragments were separated by chromatography on CM-cellulose (Whatman CM-52), equilibrated in 0.125 M sodium acetate buffer, pH 4.3 and eluted with a linear gradient of NaCl (0–1 M) in the same buffer. The peptides were desalted by gel filtration on Sephadex G-10, equilibrated and eluted in 0.01 N HCl.

The maleylation of histone H_5 was carried out at pH 9.0 and 0°C in 0.1 M K_2HPO_4 with a ten-fold molar excess of maleic anhydride per mol of free aminogroup. After one hr, the maleylated protein was exhaustively dialyzed against 0.1 M NH_4HCO_3 buffer at pH 8.0, and hydrolyzed by TPCK-treated trypsin for 4 hr at pH 8.0 and 37°C using an enzyme/substrate ratio of 1:50. After demaleylation [11] the tryptic peptides were fractionated on Chromobeads P column (Technicon) with pyridine formate and pyridine acetate buffers [12].

Chymotryptic hydrolysis of histone H_5 was carried out at 37°C for 1 hr in 0.1 M NH_4HCO_3 buffer, pH 8.0 with an enzyme substrate ratio of 1:50. The chymotryptic hydrolysate was fractionated on Sephadex G-25 F, equilibrated and eluted with 0.01 N HCl. Further purification of tryptic and chymotryptic peptides was achieved either by paper chromatography or paper electrophoresis or both methods, as described previously [13].

The Edman degradation of tryptic and chymotryptic peptides was performed manually using Sequanal grade reagents (Pierce). The Edman degradation was used either with direct identification of the phenylthiohydantoin (PTH) derivative [14], or in association with the dansylation method of Gray and Hartley [15]. In some cases, the removed residue was identified by amino acid analysis of the remaining peptide.

PTH-histidine and PTH-arginine were identified by high voltage electrophoresis (2 200 V, for one hr) on Whatman 3 MM paper at pH 3.6 (pyridine–acetic acid–water, 1:10:289, v/v), followed by specific staining respectively with the Pauly reagent and the phenanthrene quinone reagent [16]. The dansyl-amino acids were separated as described by Stehelin and Duranton [17].

3. Results and discussion

The amino acid composition of histone H_5 is shown in table 1. Based on phenylalanine as one residue, the total number of amino acid residues present in the protein is 197. The data supported by the sequence studies establish without ambiguity the presence of three tyrosines and of three histidines. Moreover, the calculated mol. wt (21 450) is now quite comparable to the values (close to 21 000), obtained with light scattering [4], sedimentation equilibrium [18] and polyacrylamide gel electrophoresis in SDS [19], and unpublished observations of M. Champagne. This result is not in agreement with our previous calculations [9,20] nor with that of Greenaway [7].

The cyanogen bromide cleavage of the protein, which contains only one residue of methionine, released two fragments CN–1 and CN–2, of which the amino acid compositions appear in table 1. The sequence of the fragment CN–1 which constitutes the N-terminal part of the histone molecule has been established by Greenaway and Murray [6]. The fragment CN–2 lacking homoserine is obviously the C-terminal part of histone H_5. The automated Edman degradation allowed us to carry the sequence determination on that fragment through 21 steps (table 2).

The limited results obtained after the cyanogen bromide cleavage of histone H_5 led us to cleave the three tyrosyl bonds of the protein with *N*-bromosuccinimide. Four peptides were obtained and separated on CM-Cellulose. The peptide in C-terminal position in the protein can easily be identified by its low absorbance at 260 nm, since it does not contain the dienone spirolactone resulting from the action of the NBS on the tyrosine. The amino acid composition of this peptide (peptide NB–4) is given in table 1. The N-terminal sequence of the NB–4 was determined as Lys–Val–Gly. The automated Edman degradation could not be carried on beyond the glycyl residue. This blocking is likely due to the bromation of the histidyl residue which comes next after the residue Gly, as demonstrated by sequence work on the chymotryptic peptide Ch–7 (table 2). However, by heating the fragment NB–4 at 80°C for 1 hr, a partial cleavage of the histidyl bond occurs. Seven steps of the manual Edman degradation, on that heated fragment allowed us to establish the N-terminal sequence (12 residues) of the peptide NB–4. This sequence was confirmed with sequence work on chymotryptic peptide Ch–7 (see below). In the tryptic hydrolysate of the maleylated histone we have identified five peptides Tm–1, Tm–2, Tm–3,

Table 1
Amino acid composition of the chicken erythrocyte histone H_5 and of its CN- and NB- fragments[a]

	Histone H_5		Fragments		
	mol/100 mol	Calculated residues[b]	CN–1[c]	CN–2 Calculated residues[b]	NB–4
Asp	1.51	3.02	0	3.13 (3)	3.03 (3)
Thr	3.02	6.02 (6)	2	4.13 (4)	4.08 (4)
Ser	11.89	23.71 (24)	6	21.23 (21)	16.55 (17)
Glu	3.83	7.65 (7)	2–3	5.2 (5)	2.54 (3)
Pro	7.84	15.64 (15)	4	9.87 (10)	9.89 (10)
Gly	4.95	9.87 (10)	0	9.1 (9)	7.22 (7)
Ala	15.86	31.62 (31)	4	25.27 (25)	23.65 (23)
Val	4.18	8.18 (8)	2	5.68 (6)	5.88 (6)
Met	0.44	0.87 (1)	1	0	0
Ile	3.03	6.05 (6)	0	4.98 (5)	2.15 (2)
Leu	4.30	8.57 (8)	2	6.3 (6)	5.79 (6)
Tyr	1.47	2.93 (3)	1	1.93 (2)	0
Phe	0.53	1.00 (1)	0	1.15 (1)	1.00 (1)
Lys	24.61	49.06 (49)	3	45.37 (45)	42.05 (42)
His	1.50	2.99 (3)	1	1.97 (2)	d
Arg	11.03	22.00 (22)	3–2	21.46 (21)	17.25 (17)
	99.99	197	31	165	142
Lys/Arg		2.23			
Basic/acidic		7.4	3.5	8.5	10
N-terminal		Blocked[e]	Blocked	Ile	Lys
C-terminal		Lys	Hse	Lys	Lys

[a] CN- and NB- fragments obtained respectively by cyanogen bromide and *N*-bromosuccinimide cleavage of the protein.
[b] Number in parentheses is the nearest integer.
[c] Data taken from references [6] and [9].
[d] The histidyl residue is chemically modified during the NBS-cleavage and is not recovered in the amino acid analysis.
[e] However 15% of the molecules are found to have threonine as the N-terminal amino acid.

Tm–4 and Tm–5* (table 2), which occur in the N-terminal part of the fragment CN–2. Tm–3 (16 residues), which contains the methionyl residue, overlaps the fragments CN–1 and CN–2. The placement of the peptides Tm–4 and Tm–5 is consistent with the data provided by the automated Edman degradation of the fragment CN–2.

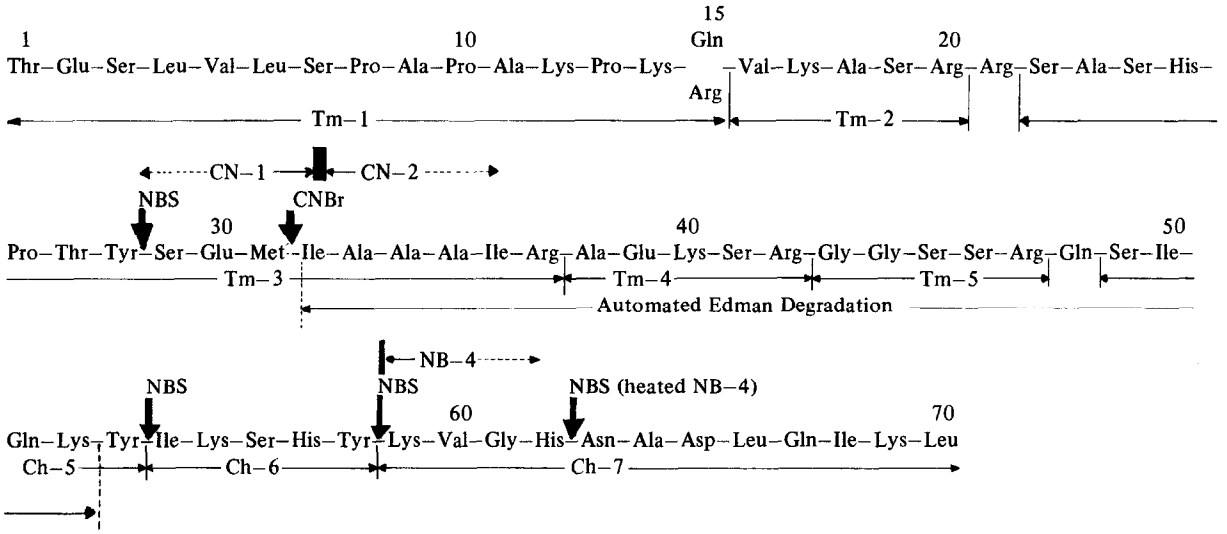
In the one-hour chymotryptic hydrolysate of the histone H_5 we have identified three peptides Ch–5, Ch–6 and Ch–7, which allowed us to extend the N-terminal sequence of the protein up to the residue 70 (table 2). Since the peptide Ch–5, Ser–Ile–Gln–Lys–Tyr, overlaps a part of the sequence of the fragment CN–2 established with the automated Edman degradation, it must be placed as written.

The peptide Ch–6, Ile–Lys–Ser–His–Tyr, contains the third tyrosyl residue of the histone molecule. Its position, adjacent to the peptide Ch–5 was deduced from the comparison between the amino acid compositions of fragments CN–2 and NB–4.

The N-terminal sequence of the peptide Ch–7 has

* These peptides are numbered according to their position in the sequence of the protein.

Table 2
N-terminal sequence of chicken erythrocyte histone H_5



been determined as Lys–Val–Gly–His and is identical to that of the fragment NB–4. The position of the peptide Ch–7, next after the tyrosine 58, is therefore obvious. The C-terminal sequence was established with the data provided by the hydrolysis of the peptide Ch–7 with the carboxypeptidases A and B.

The results that we have reported here give a general idea of the whole primary structure of the chicken erythrocyte histone H_5, in which the basic residues are concentrated in the C-terminal part of the molecule, whereas the N-terminal part of the molecule is rather hydrophobic. A similar distribution of the basic and hydrophobic residues is also found in the histone H_1 from different species [21,22].

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References

[1] Neelin, J. M., Callahan, P. X., Lamb, D. C. and Murray, K. (1964) Can. J. Biochem. 42, 1743–1752.
[2] Hnilica, L. S. (1964) Experientia 20, 13–16.
[3] Mazen, A. and Champagne, M. (1972) Biochimie 54, 1273–1279.
[4] Champagne, M. and Mazen, A. (1967) C.R. Acad. Sci. Paris, 265, 2100–2103.
[5] Champagne, M., Mazen, A. and Wilhelm, X. (1968) Bull. Soc. Chim. Biol. 50, 1261–1272.
[6] Greenaway, P. J. and Murray, K. (1971) Nature New Biology 229, 233–238.
[7] Greenaway, P. J. (1971) Biochem. J. 124, 319–325.
[8] Leboy, P. S., Cox, E. C. and Flaks, J. G. (1964) Proc. Natl. Acad. Sci. Wash. 52, 1367–1374.
[9] Garel, A., Burckard, J., Mazen, A. and Champagne, M. (1972) Biochimie 54, 451–455.
[10] Ramachandran, L. K. and Witkop, B. (1967) in: Methods in Enzymology (Hirs, C. H. W., ed.) Vol. 11, p. 283, Academic Press, New York.
[11] De Lange, R. J., Fambrough, D. M., Smith, E. L. and Bonner, J. (1969) J. Biol. Chem. 244, 319–334.
[12] Sautière, P., Tyrou, D., Laine, B., Mizon, J., Ruffin, P. and Biserte, G. (1974) Eur. J. Biochem. 41, 563–576.
[13] Sautière, P., Moschetto, Y., Dautrevaux, M. and Biserte, G. (1970) Eur. J. Biochem. 12, 222–226.
[14] Han, K., Planchon, B., Dautrevaux, M. and Biserte, G. (1973) Ann. pharm. franç. 31, 539–550.

[15] Gray, W. R. and Hartley, B. S. (1963) Biochem. J. 89, 379–380.
[16] Yamada, S. and Itano, H. A. (1966) Biochim. Biophys. Acta 130, 538–540.
[17] Stehelin, D. and Duranton, H. (1969) J. Chromatog. 43, 93–102.
[18] Diggle, J. H. and Peacoke, A. R. (1971) FEBS Lett. 18, 138–140.
[19] Panyim, S. and Chalkley, R. (1971) J. Biol. Chem. 246, 7557–7560.
[20] Champagne, M., Pouyet, J., Ouellet, L. and Garel, A. (1970) Bull. Soc. Chim. Biol. 52, 377–389.
[21] Rall, S. C. and Cole, R. D. (1971) J. Biol. Chem. 246, 7175–7190.
[22] Jones, G. M. T., Rall, S. C. and Cole, R. D. (1974) J. Biol. Chem. 249, 2548–2553.