COVALENT STRUCTURE OF THE SEA URCHIN HISTONE H_4

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1. Introduction

The primary structure of the histone H_4 has been determined in many species and shown to be highly conservative throughout evolution [1–5].

However, in the Echinoderms, the histone H_4 is characterized by the presence of one residue of cysteine [6–10]. In the histone H_4 from the sea urchin *Parechinus angulosus* [10], the residue of cysteine was identified at position 73 in place of a residue of threonine as found in calf histone H_4.

In this paper, the complete amino acid sequence of histone H_4 from the sea urchin *Psammechinus miliaris* is presented.

The primary structure was deduced from the data provided by the tryptic peptides from the oxidized and maleylated protein and by the acetic acid fragments of the protein. The sea urchin histone H_4 was found to be acetylated at a lesser extent than calf histone H_4 at the residue of lysine 16.

2. Materials and methods

Gonads of the sea urchin *Psammechinus miliaris* were frozen in solid CO_2 after excision and kept at −20°C until use.

Chromatin was obtained either from purified nuclei as described previously [9] or directly from the gonads according to the procedure of Strickland et al. [10]. The F_{2a} histone fraction was extracted from the chromatin and subsequently fractionated in F_{2a1} and F_{2a2} subfractions as described by Johns [11]. The histone H_4 was isolated from the F_{2a1} fraction by gel filtration chromatography on Biogel P 60 column equilibrated and eluted with 0.01 N HCl–0.05 M NaCl–0.02% NaN_3.

The purity of the protein was assessed by electrophoresis in polyacrylamide gel at pH 2.7 according to Panyim and Chalkley [12] using a 17% acrylamide concentration.

The amino acid composition was established on 24 h and 72 h hydrolysates.

After performic acid oxidation according to Hirs [13], the histone was maleylated and hydrolyzed with TPCK-treated trypsin for 4 h at pH 8.0 and 37°C in 0.1 M NH_4HCO_3, using an enzyme/substrate ratio of 1:50.

After demaleylation [1] the tryptic peptides were fractionated on Chromobeads P column (Technicon) with pyridine–formate and pyridine–acetate buffers [14].

Cleavage of aspartyl bonds by 0.25 N acetic acid was performed at 105°C for 6 h in sealed evacuated tubes (10 mg of protein per ml of acetic acid). The cleavage products were fractionated on Sephadex G 50 F column equilibrated and eluted with 0.01 N HCl.

Structural studies of the peptides were carried out as described previously [3].

For the determination of ϵ-*N*-acetyllysine peptides were hydrolyzed with aminopeptidase M for 24 h at 40°C and pH 8.0 in 0.1 M NH_4HCO_3 (0.5 mg of enzyme per μmole of peptide). The hydrolysates were subsequently analyzed on a Beckman Multichrom amino acid analyzer.

Abbreviation: TPCK, L-(1-tosylamido-2-phenyl)-ethyl chloromethyl ketone.

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N-ACETYL-SER-GLY-ARG-GLY-LYS-GLY-GLY-LYS-GLY-LEU-GLY-LYS-GLY-GLY-ALA-LYS(AC)-ARG-HIS-ARG-
          1                                        10

LYS(ME)-VAL-LEU-ARG-ASP-ASN-ILE-GLN-GLY-ILE-THR-LYS-PRO-ALA-ILE-ARG-ARG-LEU-ALA-ARG-ARG-
                                                 30                                    40

GLY-GLY-VAL-LYS-ARG-ILE-SER-GLY-LEU-ILE-TYR-GLU-GLY-THR-ARG-GLY-VAL-LEU-LYS-VAL-PHE-LEU-
                                                                                    60

GLU-ASN-VAL-ILE-ARG-ASP-ALA-VAL-THR-TYR-CYS-GLU-HIS-ALA-LYS-ARG-LYS-THR-VAL-THR-ALA-MET-
                                   70          73                                  80

ASP-VAL-VAL-TYR-ALA-LEU-LYS-ARG-GLN-GLY-ARG-THR-LEU-TYR-GLY-PHE-GLY-GLY-COOH
                    90                                          100      102
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Fig.1. Complete amino acid sequence of the sea urchin histone H_4.

3. Results and discussion

The peptides identified in the soluble and insoluble fractions of the tryptic hydrolysate of the oxidized and maleylated histone H_4 correspond to 100 of the 102 residues present in the protein molecule of which the complete amino acid sequence is given in fig.1. The cleavage of two Arg–Arg bonds, produces free arginine which accounts for the two lacking residues.

Each of these peptides has its homologue in the tryptic hydrolysate of the maleylated histone H_4 from calf thymus except peptide from residue 68 through 78 which contains the only residue of cysteine present in the sea urchin histone H_4.

The residue of cysteine was identified in position 73 as cysteic acid. In the calf histone H_4, a residue of threonine is found in this position.

The residue of lysine in position 16 was found to be acetylated to an extent of 20%. This result clearly shows the presence of both acetylated and unacetylated forms in sea urchin histone H_4 as already evidenced in polyacrylamide gel electrophoresis. The acetylated form has not been found in the histone H_4 of the sea urchin *Parechinus angulosus* [10].

The acetic acid cleavage of three aspartyl bonds at residues 24, 68 and 85 in the histone H_4 yields five peptides which are separated on Sephadex G 50 F (fig.2). These peptides, characterized by their amino acid composition (table 1) and their amino terminal sequence, were ordered by comparison with the amino acid sequence of the calf histone H_4.

These large peptides are of great interest not only in the determination of the primary structure of the protein by providing overlappings to the tryptic

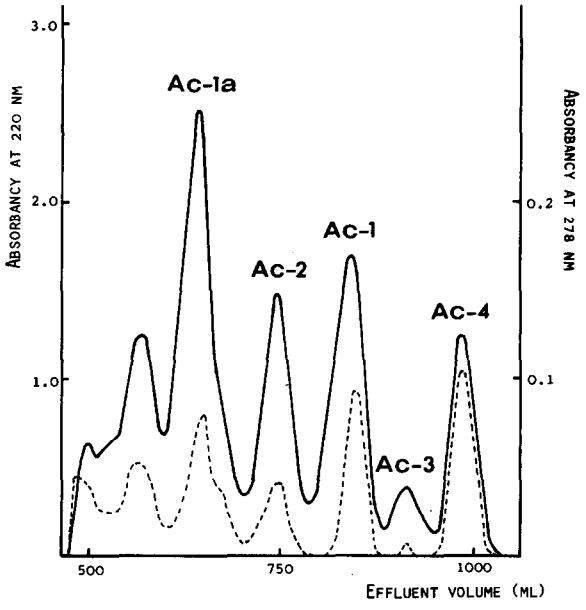

Fig.2. Separation by gel filtration chromatography on Sephadex G 50 F equilibrated and eluted with 0.01 N HCl of the peptides obtained by acetic acid cleavage of the sea urchin histone H_4.

Table 1
Amino acid composition of the sea urchin histone H_4 and of the peptides obtained by acetic acid cleavage of the protein

Amino acid[a]	H_4	Ac-1 (1–24)	Ac-2 (25–67 26–68)	Ac-1a (1–67)	Ac-3 (69–85)	Ac-4 (86–102)
Aspartic acid	5	1.05 (1)	2.63 (2)	3.08 (3)	0.85 (1)	
Threonine	6		2.74 (2)	2.42 (2)	2.65 (3)	0.99 (1)
Serine	2	0.95 (1)	0.91 (1)	2.38 (2)		
Glutamic acid	6		4.02 (4)	3.52 (4)	0.92 (1)	0.73 (1)
Proline	1		0.98 (1)	1.19 (1)		
Glycine	17	7.92 (8)	5.15 (5)	13.15 (13)		3.71 (4)
Alanine	7	1.33 (1)	3.11 (3)	4.74 (3)[b]	2.85 (3)	1.06 (1)
Cysteine	1				not det.	
Valine	9	1.02 (1)	4.05 (4)	3.82 (5)[b]	2.04 (2)	1.35 (2)
Methonine	1				0.90 (1)	
Isoleucine	5–6		4.65 (6)[b]	4.73 (6)[b]		
Leucine	8	1.98 (2)	3.97 (4)	5.11 (6)[b]		1.95 (2)
Tyrosine	4		1.48 (1)	1.00 (1)	0.87 (1)	1.95 (2)
Phenylalanine	2		1.11 (1)	1.00 (1)		0.98 (1)
Lysine	10	4.95 (5)	3.69 (3)[b]	8.59 (8)	2.11 (2)	1.07 (1)
Histidine	2	1.05 (1)		1.22 (1)	0.95 (1)	
Arginine	14	3.66 (4)	6.82 (7)	10.18 (11)	1.33 (1)	1.99 (2)
ε-Me-Lysine	1					

[a] Amino acid values are expressed as molar ratios.
[b] Values obtained from structural studies.

peptides but also to determine the regions of the amino acid sequence of the protein which are involved in hydrophobic or ionic interactions.

The peptide Ac-1a (residues 1 through 67) results from a partial cleavage at residue 24. The partial deamidation of the asparaginyl residue at position 25 gives rise to a fourth aspartyl bond. The cleavage of the two adjacent aspartyl bonds at positions 24 and 25 yields a mixture of two peptides of 43 residues (peptides Ac-2) starting respectively at residues 25 and 26 as evidenced by four cycles of the Edman degradation. Indeed, the amino terminal sequences,

Asn–Ile–Gln–Gly and Ile–Gln–Gly–Ile
25 26

were determined simultaneously in that mixture.

The composition of the peptide Ac-1 is characteristic of the amino terminal sequence of the histone H_4: presence of acetylated and methylated lysine, high content of glycine. Moreover, this peptide is lacking of a free aminogroup. Therefore it must be the amino terminal peptide of the histone molecule.

The peptide Ac-2 overlaps five tryptic peptides from the maleylated protein. The presence of two extra arginine in its composition has to be related to the two Arg–Arg bonds at positions 35–36 and 39–40 by comparison with the amino acid sequence of the calf histone H_4.

The peptide Ac-3 (residues 69 through 85) contains the only residue of cysteine and the only residue of methionine present in protein. Its amino terminal sequence was determined as Ala–Val–Thr–Tyr. It overlaps tryptic peptides Tm-10 and Tm-11.

The peptide Ac-4 (residues 86 through 102) is characterized by the presence of two tyrosyl residues, of one phenylalanyl residue and the absence of an aspartyl residue. Its amino terminal sequence was determined as Val–Val–Tyr–Ala. This peptide is obviously the carboxyterminal peptide of the histone molecule.

Thus, the amino acid sequence of the sea urchin histone H_4 differ, only from that of the calf histone H_4 by the replacing of a threonyl residue by a cysteinyl residue at position 73. This mutation occurs in the hydrophobic region of the protein from

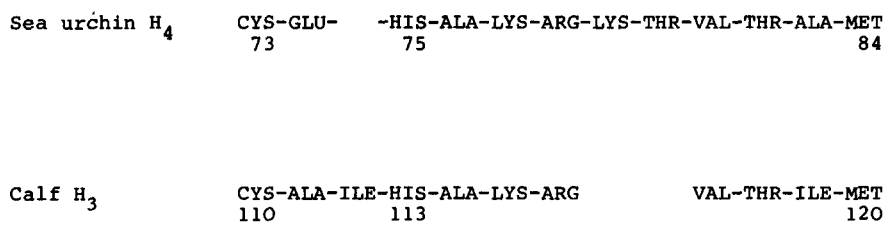

Fig.3. Sequence homology in the vicinity of the cysteinyl residue between sea urchin histone H_4 and calf histone H_3.

residues 50 through 75, region which is involved in histone–histone interactions.

The presence of cysteine in the sea urchin histone H_4 is a very interesting feature which brings out a remarkable sequence homology between the histones H_3 and H_4 (fig.3), already quoted by Strickland et al. [10].

This homology might be the reflection either of a common origine from the same ancestral gene or of an analogy of function in the chromatin or both.

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