COMPARISON OF THE N-TERMINAL AMINO ACID SEQUENCES OF HISTONE F3 FROM A MAMMAL, A BIRD, A SHARK, AN ECHINODERM, A MOLLUSC AND A PLANT.

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1. Introduction

It has often been stated that histone F2al (IV) and F3 (III) are evolutionarily extremely stable proteins [1–3,5]. This was based on the observation that these histones had a virtually identical electrophoretic mobility regardless of the source [1,2]. The tryptic fingerprint maps of the two proteins from calf and pea were shown to be very similar [5] and in the case of histone F2al from pea and calf the primary structures had been established and found to differ by only two amino acid residues out of a total of 102 residues [6]. The assumption of evolutionary constancy of F3 throughout the biological world is mainly based on electrophoretic studies [1,2]. Only recently the sequences of histone F3 isolated from calf [7], chicken [8,9] and carp [4] have been established. The latter two were found to be identical but differed in their sequence from the calf histone with respect to residue Ser 96 which is replaced by Cys in calf.

In order to establish the evolutionary relationships of histone F3 over a more extended range in the biological world we isolated this histone from a number of organisms separated widely on the evolutionary scale, namely from a shark, an echinoderm, a mollusc and a plant. Their electrophoretic mobility, amino acid composition and the N-terminal 40–50 amino acid sequence were compared to those of histone F3 from calf [7] and chicken [8,9].

2. Experimental

Nucleoprotein from chicken and shark erythrocytes was isolated as reported previously [10]. Nucleoprotein from sea urchin and mollusc male gonads were isolated by suspending the cells in 0.14 M NaCl–0.01 M Na citrate–0.01 M $NaHSO_3$–0.2%. Triton X 100® followed by homogenization in a Dounce homogenizer and centrifugation at 10 000 *g* for 10 min. The pellet was washed 6 times by repeating the above steps but omitting the Triton in the wash medium.

Pollen grains from cycad were suspended in 0.1 M NaCl–0.01 Na citrate–0.01 M $NaHSO_3$–0.01 M EDTA (wash medium) and ruptured in a cell homogenizer (model MSK B. Braun (II)) set at full speed for 2 min using 0.5 mm glass balls followed by centrifugation at 10 000 *g* for 10 min. The cell debris was washed 6 times by repeated homogenization in the wash medium with an Ulta-Turrax homogenizer (Janke & Kunkel KG) and centrifugation at 15 000 *g* for 10 min. The pellet was finally extracted with 2 M NaCl and the clear viscous supernatant poured slowly into distilled water (final NaCl conc. 0.2 M) and the nucleoprotein removed by winding it around a glass rod.

Crude F3 histones were isolated from the nucleoprotein of all organisms by the method of Johns [10,12]. F3 histones were purified by gel filtration as reported previously [10]. Proteins were hydrolysed in 5.7 N HCl at 110°C for 24 hr. Phenol was added as an anti-oxidant and hydrolysis tubes were flushed with N_2 and evacuated to below 0.02 Torr.

Sequence analyses were performed on a Beckman 890 sequencer. Instead of the Quadrol® [14], 3-dimethylamino-1 propyne buffer [15] was used. The protein degradation program as provided by the manufacturer was modified to allow for the higher

volatility of this buffer, i.e. several additions of buffer were made during the coupling step and the ethyl acetate wash step was shortened.

PTH-amino acids were identified by gas chromatography [16] and amino acid analysis after rehydrolysis of the derivatives in 5.7 N HCl–1% thioglycollic acid for 24 hr at 130°C [17].

Amino acid analyses were performed on a Beckman model 116. For the determination of the amino acids generated from the thiohydantoins the analyser was modified to allow the detection of 3 nmoles amino acid. The methylated lysine species appeared as a single peak between Lys and His.

3. Results and discussion

Histone F3 (III) fractions were isolated from various tissues of a number of organisms widely separated on the evolutionary scale, namely shark erythrocytes (*Poroderma africanum*), sea urchin sperm (*Parechinus angulosus*) mollusc sperm (*Patella granatina*) and cycad pollen (*Encephalartos caffer*). The purification procedure for histone F3 developed previously [10] involved the removal of large molecular weight proteins from the reduced crude histone F3 preparation by gel filtration. Histone F3-containing fractions were pooled and oxidized with iodosobenzoic acid [10] followed by rechromatography on the same column. This procedure yielded a pure histone F3 dimer from all the organisms mentioned above. All the dimers possessed an identical electrophoretic mobility (fig. 1). This was ascertained by co-electrophoresis of the chicken histone F3 with each of the other fractions.

The electrophoretic pattern of these histones after the reduction of the dimer with mercaptoethanol is shown in fig. 2, and again reveals their closely related

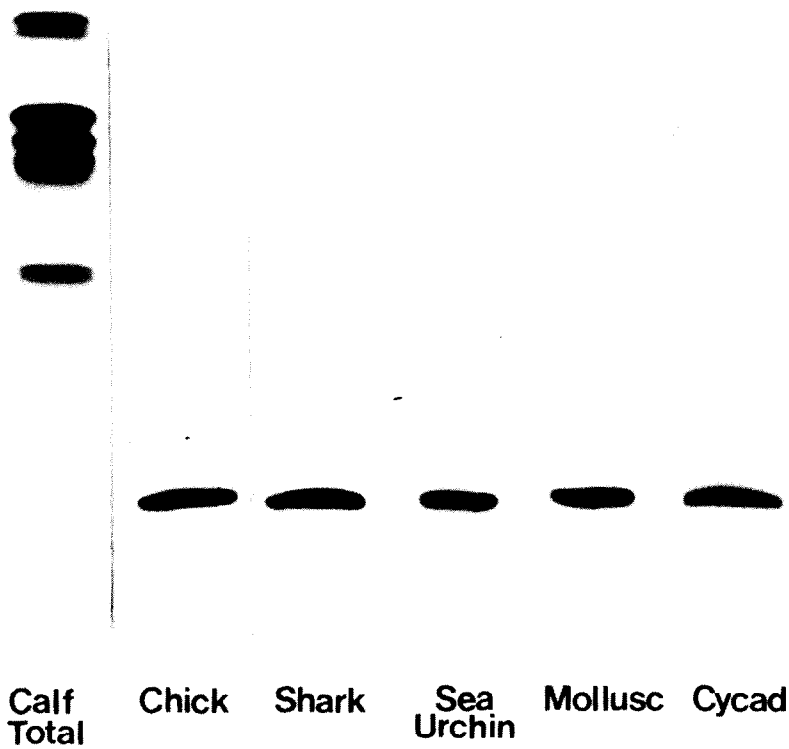

Fig. 1. Comparison of the electrophoretic mobility of histone F3 dimer from various organisms to total calf thymus histones. The F3 dimers were isolated from a Sephadex G-100 column after the oxidation of the crude preparation with iodosobenzoate. All gells contained 15% acrylamide and were run for 3.5 hr.

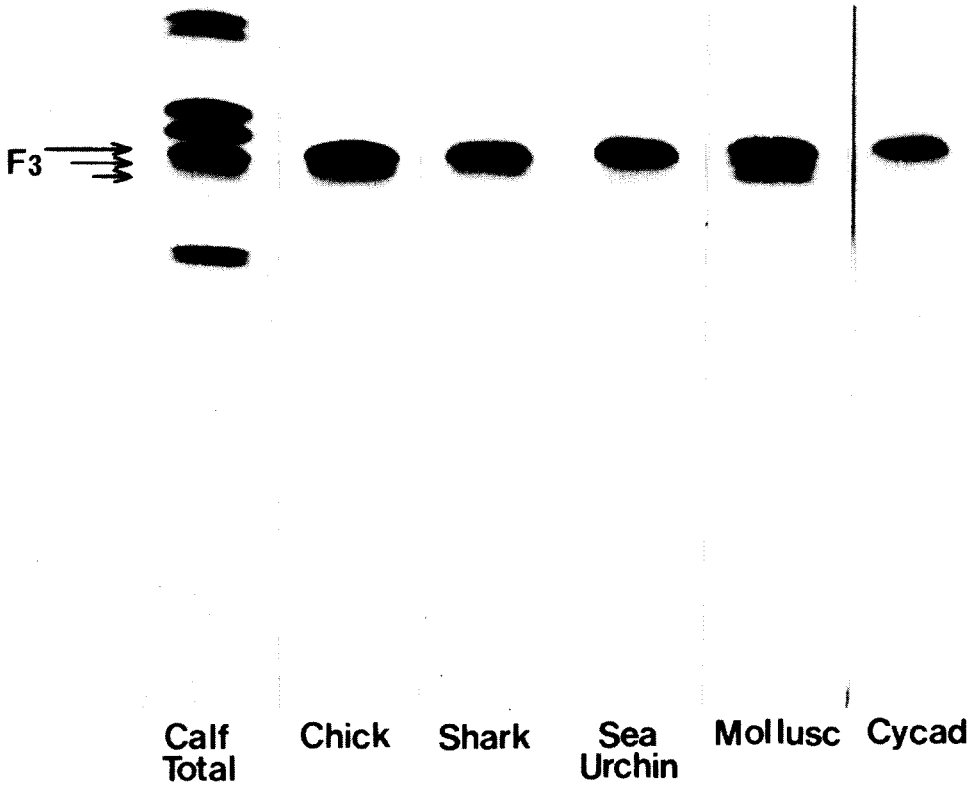

Fig. 2. Comparison of the elctrophoretic mobility of histone F3 obtained after the reduction of the dimer with mercaptoethanol.

electrophoretic mobility. It is apparent that the microheterogeneity due to acetylation of lysine residues resulting in the loss of one positive charge per acetyl residue and corresponding loss in electrophoretic mobility [10,19,20] varied among the histones (fig. 2). Calf, chicken, shark and mollusc all possessed at least the mono- and diacetylated protein. Sea urchin F3 histone is only to a very small extent acetylated while in cycad only the unacetylated protein could be detected.

The amino acid composition of all four histones is given together with that of calf and chicken histone F3 in table 1.

All the F3 histones were subjected to between 40 and 50 automatic Edman degradations. Except for a single replacement of Tyr-41 by Phe in the cycad histone (fig. 3) the gas chromatograms and the amino acid composition of a particular degradation cycle were indistinguishable for the four histones indicating that their N-terminal sequences were identical (fig. 3).

From fig. 3 it is evident that the extent of ϵ-N-methylation of certain lysine residues differs from organism to organism. Notably Lys-9 and -27 are always partly or completely methylated (fig. 3). Lys-36 in cycad is not methylated to a detectable extent which also seems to be the case in calf [7]. In contrast to the other histones Lys-5 in cycad is also partly methylated.

It must be pointed out that the ratio of mono- to di- to trimethyl lysine was not determined and that this ratio may vary for the various methylated Lys residues, depending on the functional state of the cells from which the histones were isolated.

Quite early in our investigation of the primary structure of histone F3 from chicken [8] it became apparent that nearly over the whole protein molecule a repetitive unit consisting generally of nine residues of which the last two are basic residues can be recognized. This repetitive pattern occurs four times in the N-terminal regions compared in fig. 3.

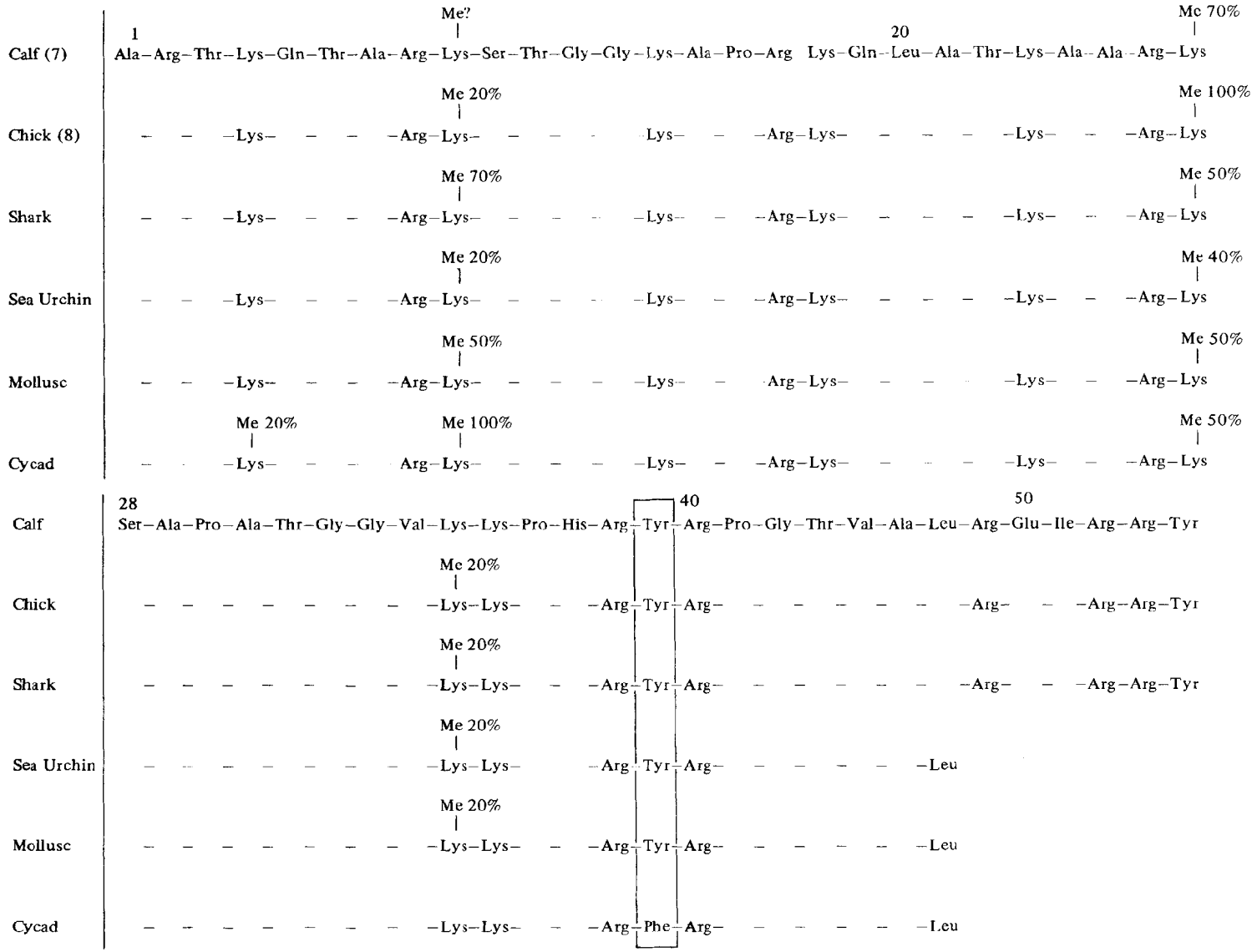

Fig. 3. N-terminal amino acid sequence of histone F3 from chicken, shark, sea urchin, mollusc and cycad determined by automated Edman degradation. Sites of ε-N-methylation are indicated by Me followed by the approximate amount present expressed as a % total. The sequence of calf histone F3 has been determined by DeLange et al. [7].

Table 1
Amino acid composition of F3 histones from various organisms

	Calf	(18)	Chicken		Shark		Sea Urchin		Mollusc		Cycad	
Lys	9.0	(13)	8.47	(13)	9.07	(13)	9.65	(13)	9.60	(13)	9.10	(13)
ε-*N*-MeLys	1.0		1.39		0.87		0.56		0.69		1.34	
His	1.7	(2)	1.49	(2)	1.41	(2)	1.53	(2)	1.50	(2)	1.66	(2)
Arg	13.0	(18)	13.41	(18)	13.68	(18)	12.95	(18)	13.40	(18)	13.41	(18)
Asp	4.2	(5)	3.73	(5)	3.69	(5)	3.80	(5)	3.90	(5)	3.78	(5)
Thr	6.8	(10)	6.66	(10)	6.74	(10)	6.23	(10)	6.42	(10)	6.48	(10)
Ser	3.6	(5)	3.92	(6)	3.74	(6)	4.25	(6)	4.00	(6)	3.18	(5)
Glu	11.5	(15)	11.19	(15)	11.51	(15)	11.96	(15)	11.49	(15)	11.25	(15)
Pro	4.6	(6)	4.6	(6)	4.74	(6)	4.65	(6)	4.53	(6)	4.56	(6)
Gly	5.4	(7)	5.56	(7)	5.54	(7)	5.88	(7)	5.64	(7)	5.60	(7)
Ala	13.3	(18)	13.51	(18)	13.60	(18)	13.33	(18)	13.46	(18)	14.49	(19)
½ Cys	4.0	(2)	0.54	(1)	0.62	(1)	0.65	(1)	0.70	(1)	0.55	(1)
Val	4.4	(6)	4.49	(6)	4.36	(6)	4.67	(6)	4.60	(6)	4.69	(6)
Met	1.1	(2)	1.34	(2)	1.36	(2)	1.43	(2)	1.39	(2)	0.69	(1)
Ile	5.3	(7)	5.07	(7)	5.14	(7)	4.87	(7)	4.93	(7)	4.74	(7)
Leu	9.1	(12)	8.89	(12)	8.77	(12)	8.62	(12)	8.76	(12)	8.91	(12)
Tyr	2.2	(3)	2.24	(3)	2.18	(3)	2.21	(3)	2.25	(3)	1.60	(3)
Phe	3.1	(4)	2.92	(4)	2.92	(4)	3.11	(4)	2.89	(4)	3.86	(5)
NH_3	7.0		8.59		8.28		11.60		8.90		9.15	

The amino acids are given as mole per 100 moles recovered. No corrections have been made for hydrolytic losses. Cysteine has been estimated as cysteic acid [13]. Figures in parentheses are the most likely number of residues present per histone molecule. Values for chicken and calf have been calculated from sequence analysis [7–9]. Suspected changes in the composition have been underlined.

From fig. 3 it is apparent that the terminal Lys residues in three out of four repetitive peptides are generally methylated with cycad being an exception. The terminal lysine (Lys-18) in the second repeat unit was never found to be methylated, which may be due to the preceding Pro which causes drastic alteration in the secondary structure in this region. Additionally equivalent Lys residues in the repeat unit are acetylated in calf thymus [7] and possibly also in the other organisms.

The sequence identity in the N-terminal part of the molecule, except for residue 41, the very close similarity in the amino acid composition and the identity of the electrophoretic mobilities confirms the evolutionary stability of this particular histone.

The evolutionary stability of this histone may partially be determined by the surface topography of the DNA with respect to the spacing of phosphate groups and the hydrophobic interior of the major and minor grooves.

The repetitiveness of the arrangement of basic residues in addition may put certain constraints on the conformation of the DNA–histone complex and cause it to fold in some regular fashion of higher order, e.g. supercoil. The latter can then be stabilized by hydrophobic interaction of the non basic stretches of the histone molecule.

Acknowledgements

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