THE PRIMARY STRUCTURE OF HISTONE F3 FROM SHARK ERYTHROCYTES

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1. Introduction

The amino acid sequence of chicken histone F3 (III) isolated from erythrocytes had previously been elucidated [1–3]. This had been achieved by selective chemical fragmentation and alignment of the peptides by generating not more than 3 peptides in any single cleavage. From the identity of the N- and C-terminal residues of the cleavage products and the uncleaved starting material [2, 3] the peptides could be aligned. In order to further study the evolution of histone F3, we isolated it from shark erythrocytes. Sharks are believed to have evolved about 350 million years ago and their ancestors separated 500 million years ago from the main line of vertebrate evolution. They are therefore of considerable interest in this context. The amino acid seuqence of shark histone F3 was elucidated by the same procedure used for the chicken histone.

The sequence of this protein can now be compared to that of chicken [3], calf [5] and the recently determined carp histone [6].

2. Experimental

Shark blood (*Poroderma africanus*) was collected in 10% sodium citrate (100 ml/l of blood) with stirring and transported over ice to the laboratory. All subsequent operations were performed at between 0–4°C.

All other techniques used were the same as those reported previously [1–3].

3. Results and discussion

Histone F3 was isolated from shark erythrocytes by the same method used for chicken histone F3 [1]. The electrophoretogram of the dimer obtained after oxidation with iodosobenzoic acid and gel filtration is shown in fig. 1. After reduction with mercaptoethanol this histone shows a similar electrophoretic microheterogeneity as chicken and calf histone F3 [1] which is due to acetylation of lysine residues [7, 3].

This shark histone has an identical mobility when compared to the chicken histone F3, this was ascertained by co-electrophoresis of the two histones.

The amino acid composition of the shark histone F3 is very closely related to the corresponding fraction from chicken (table 1).

The approach used for the elucidation of shark histone F3 was identical to that applied to the chicken histone [2, 3]. The dimer was subjected to CNBr cleavage. The three purified fragments [2] were aligned by comparing their N-terminal sequence and C-terminal amino acid of the uncleaved protein and its fragments (table 1).

Fragment CN-1 was cleaved with *N*-bromosuccinimide [8] into an additional set of three fragments (CN-1 NB-1 [2], CN-1 NB-2, CN-1 NB-1 [1], table 1) which were aligned after purification [2, 3] by the same reasoning as above.

The amino acid sequence was elucidated by subjecting the protein and fragments CN-1 NB-2, CN-1 NB-1 [1], CN-2 and CN-3 to the automatic Edman degradation. We were able to assign the first 48 residues in the uncleaved protein. In all the fragments except CN-2 it was possible to position all amino acid up to the penultimate residue. The C-terminal residue

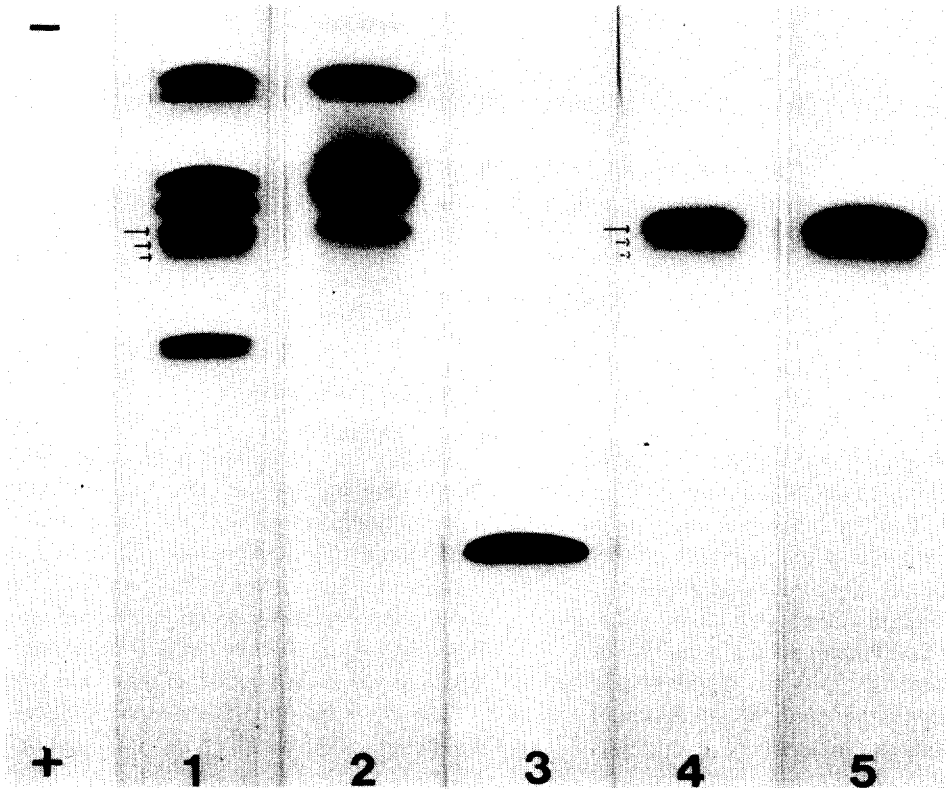

Fig. 1. Electrophoretic pattern of whole calf thymus histone [1]. whole shark histone [2], shark histone F3 dimer [3], the dimer after reduction with mercaptoethanol [4] and shark histone F3 together with chicken histone F3 [5]. All gels contained 15% acrylamide and were run for 3.5 hr [1]. (Arrows indicate unacetylated, mono- and diacetylated species of F3 histone.)

was inferred from the amino acid composition of the fragments.

In fragment CN-2 drastic reduction in the yields of phenylthiohydantoins at the sequence Glu-Gln and Asp-Thr-Asn occurred. We therefore cleaved CN-2 at the Asp residues with 0.03 N HCl for 12 hr at 105°C [9] and subjected the peptides to the Edman degradation. In addition fragment CN-2 was cleaved with trypsin and from the unseparated mixture the C-terminal sequence could be established by the sequential degradation. We were then able to position all amino acid in this histone.

In the automated Edman degradation a certain amount of ambiguity exists as to whether dicarboxylic amino acids exist in the free or amide form. This ambiguity becomes marked in later stages of a degradation and we have therefore left some dicarboxylic acids unassigned (residue 73 Glx, 76 Glx, 77Asx, 81 Asx, 85 Glx). Lysine 9,27 and 36 are methylated and the dimethyl derivative predominates. The same residues are methylated in chicken histone [3]. The presence or absence of the trimethyl derivative could not be ascertained.

Judging from the gel electrophoretic pattern (fig. 1) the unacetylated form predominates over the mono- and diacetylated forms. A similar situation pertains to calf and chicken histone F3 [1]. It was not possible to position the acetyl groups but electrophoretic studies indicated that they were in the N-terminal fragment CN-1 NB-1 [2].

Table 1
Amino acid composition of shark histone F3 and fragments

	F3	CN-1	CN-1 NB-1 (2)	CN-1 NB-2	CN-1 NB-1 (1)	CN-2	CN-3
Lys	9.0 (13)	11.0 (11)	16.3 (8)	1.7	8.5 (3)	4.0 (1)	7.1 (1)
ε-*N*-MeLys	0.9	1.5	3.0	–	–	–	–
His	1.4 (2)	0.9 (1)	+ (1)	–	–	3.0 (1)	–
Arg	13.4 (18)	14.6 (13)	11.8 (5)	30.3 (4)	11.8 (4)	4.5 (1)	26.2 (4)
Asp	3.7 (5)	2.6 (2)	0.9	1.1	5.6 (2)	7.0 (2)	6.5 (1)
Thr	7.1 (10)	8.7 (8)	11.3 (5)	7.1 (1)	4.7 (2)	6.4 (2)	0.6
Ser	4.2 (6)	5.3 (5)	5.0 (2)	0.9	6.4 (3)	3.0 (1)	0.6
Glu	11.3 (15)	10.2 (9)	5.0 (2)	9.2 (1)	17.6 (6)	14.1 (4)	13.6 (2)
Pro	4.5 (6)	5.4 (5)	8.4 (3)	8.1 (1)	3.0 (1)	0.2 –	6.2 (1)
Gln	5.5 (7)	5.7 (5)	9.8 (4)	7.8 (1)	0.3 –	3.9 (1)	6.5 (1)
Ala	13.4 (18)	12.4 (11)	19.6 (8)	9.3 (1)	6.3 (2)	17.1 (5)	13.0 (2)
Cys	0.6 (1)	–	–	–	–	2.7 (1)	–
Val	4.4 (6)	4.5 (4)	3.1 (1)	7.4 (1)	5.4 (2)	6.7 (2)	0.4
Met	1.4 (2)	0.6 (1)	–	–	1.3 (1)	1.3 (1)	–
Ile	5.1 (7)	3.3 (3)	0.9	7.6	6.0 (2)	6.4 (2)	11.9 (2)
Leu	8.8 (12)	7.8 (7)	4.0 (1)	8.7 (1)	14.6 (5)	13.1 (4)	7.0 (1)
Tyr	2.2 (3)	2.2 (2)	+ (1)	+ (1)	–	3.2 (1)	0.2
Phe	2.9 (4)	3.3 (3)	1.0	0.5	8.4 (3)	3.4 (1)	0.4
NH_3	8.3	8.3	10.5	12.6	13.4	10.7	9.8
No. residues per molecule	135	90	41	13	36	30	15
N-terminal	Ala-Arg	Ala-Arg	Ala-Leu	Arg	Glx	Ala	Pro
C-terminal	Ala	Met	Tyr	Tyr	Met	Met	Ala

Amino acid composition is given as mole percent of all amino acids recovered followed by the most likely number of residues per molecule. Corrections for hydrolytic losses of 5% and 10% for Ser and Thr [1] have been made. After CNBr cleavage Met was determined as homoserine lactone. Tyr was shown to be present after NBS-cleavage by the 260 nm absorption of the spirolactone [8] and His by the Pauly spot test [12].

The amino acid sequence of chicken histone F3 published [2] has been corrected [3] and the results together with experimental details have been submitted for publication. We have now assigned in chicken histone F3 to position 59 Glu, to position 73 Glx and to position 106 Asp instead of the originally reported Gln, Gln, and Asn respectively. The Glx residue 86 could not be verified and is supernumerous. This positions the original Ser 90 into position 86 and brings the toal number of residues from 136 to 135.

The amino acid sequence of shark histone F3 is identical to that of chicken [3] and to the recently determined carp histone [4]. A slight quantitative difference in the acetylation and methylation of lysine residues seems however to exist and is probably determined by the functional state of the chromatin from which these histones have been isolated.

All three histones differ from that of calf in that Ser-96 is replaced by Cys in the latter.

This adds further evidence to the suspected conserconservative nature [10, 11] of histone F3.

Acknowledgements

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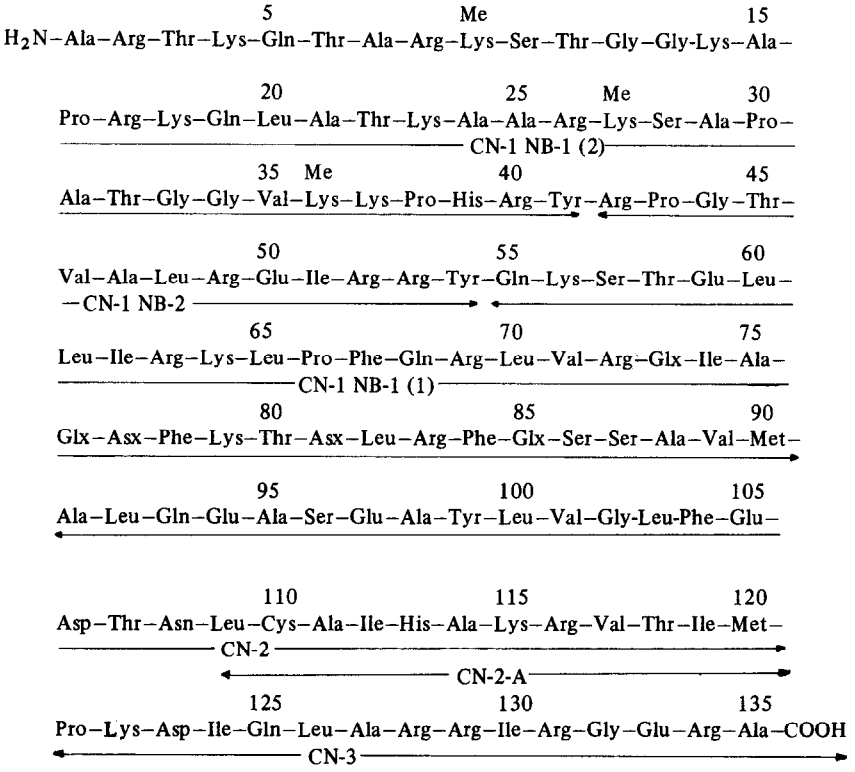

Fig. 2. Amino acid sequence of histone F3 isolated from shark erythrocytes. The extent of methylation is approximately: Lys 9:70%, Lys 27:50%, and Lys 36:20%.

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