PURIFICATION AND CHARACTERIZATION OF THE CYSTEINE-CONTAINING F3 HISTONE FROM CHICKEN ERYTHROCYTE

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1. Introduction

Knowledge of the amino acid sequence of histones will assist in the understanding of the molecular mechanisms of their function in the cell nucleus. Up to now only histones of group F3 have been shown to contain cysteine giving to this histone group particular properties with respect to protein-protein interaction. The structure of F3 histones is therefore of special interest.

A new method has been developed to isolate highly purified F3 (Arg-rich) histone from chicken erythrocytes in gram amounts. The protein was characterized by its molecular weight, amino acid composition and end groups. Evidence was obtained that the microheterogeneity of this protein is due to fractional acetylation.

The three CNBr-cleavage fragments were isolated and their relative sequence in the protein determined. Amino acid analysis of these fragments revealed clustering of acidic and basic amino acids.

2. Matcrials and methods

Nucleoprotein was isolated essentially by the method of Murray [1] modified by adding bisulphite or mercaptoethanol to the saline wash medium to prevent proteolysis [2]. Crude F3 histone was prepared from the nucleoprotein by the method 2 of Johns [3]. Total histones from calf thymus and chicken erythrocyte nucleoprotein were extracted with 0.2 N HCl [2].

Polyacrylamide gel electrophoresis was carried out according to the method of Panyim and Chalkley [4].

Amino acid analysis was performed on a Beckman Model 116 amino acid analyzer using the 4 hr procedure.

Gas chromatography was used to identify acetic acid in histone hydrolysates using a LAC-446 diatoport S column.

Further experimental details are given in the legends to figures and tables.

3. Results and discussion

A method has been developed to isolate highly purified F3 histone from chicken erythrocytes in gram amounts. Crude F3 prepared by the method 2 of Johns [3] is still contaminated by histone F2a2 (fig. 1a, gel 3). Purification of the crude F3 fraction was achieved by oxidizing the histone in this preparation with o-iodosobenzoate equivalent to its cysteine content, at pH 7 and 0° in 8 M urea. This results in the formation of dimer which could be separated from the contaminating impurities mainly histone F2a2 by gel filtration [5] (fig. 2, a, b). The degree of purification achieved is evident from the gel electrophoresis of the dimer and the monomer (fig. 1a, gel 4 and 5) which is prepared from the dimer by reduction with mercaptoethanol.

The purified chicken F3 histone shows a microheterogeneity resembling that of calf thymus F3 histone (fig. 1a, gel 1 and 5). A similar microheterogeneity is generally observed in the F2a1 fraction (fig. 1, gel 1 and 2). It was shown by DeLange et al. [6] that lysine residue 16 in this histone is 50% acetylated which is probably responsible for the formation of two bands on polyacrylamide gel elec-

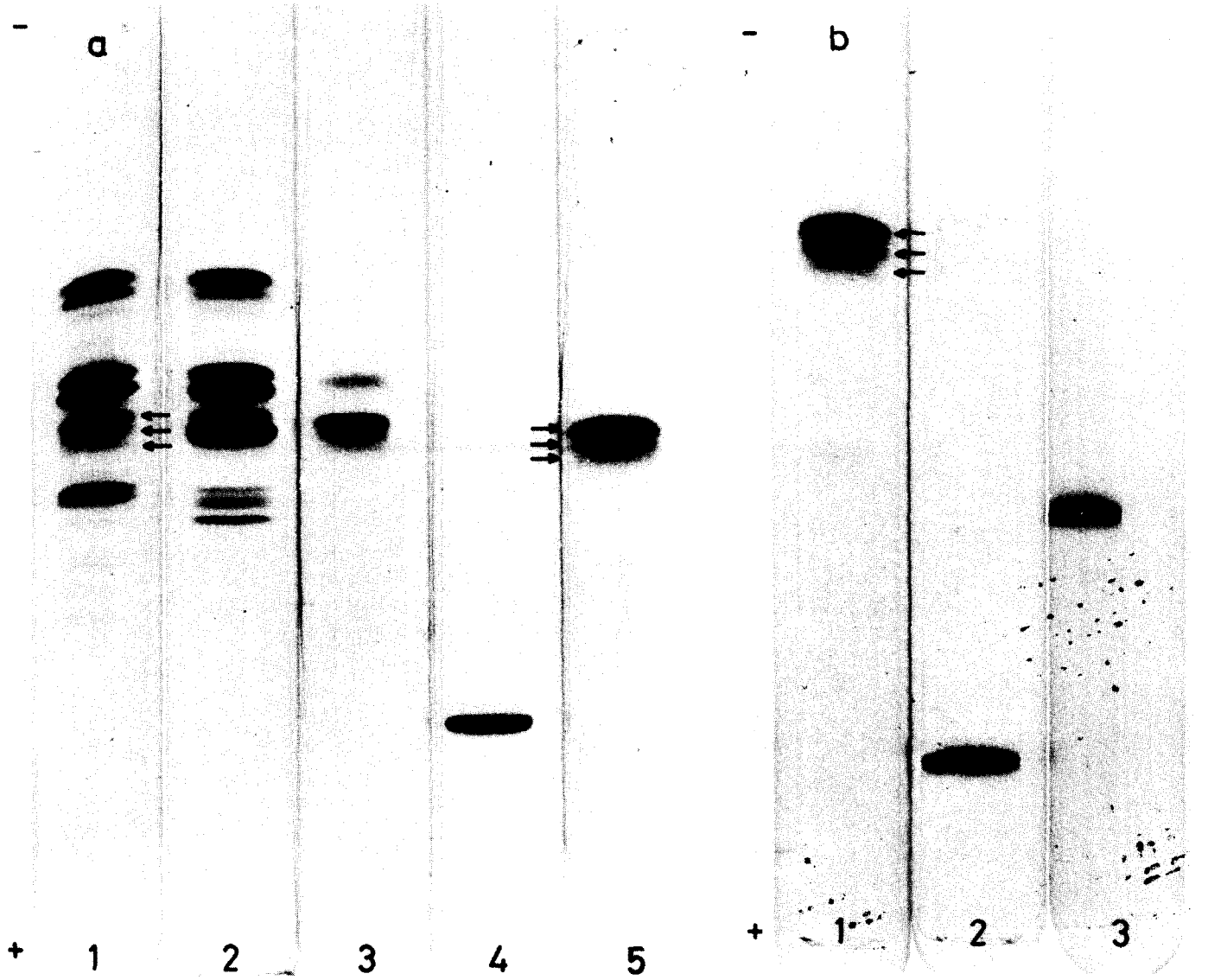


Fig. 1a. Polyacrylamide gel electrophoretic pattern of: (1) total calf thymus histones; (2) total chicken erythrocyte histones; (3) crude F3 chicken erythrocyte histones; (4) chicken erythrocyte F3 histone dimer; (5) chicken erythrocyte F3 histone obtained after reduction of the dimer with β-mercaptoethanol. The major histones in gel (1) are from top to bottom; F2a1, acetylated F2a1, F2a2, F2b, F3 (a group of three bands →) and F1 (two bands) (4) In gel (2) F3 is partly obscured by histone F5 (serine rich). Fig. 1b. Polyacrylamide gel electrophoretic pattern of the three fragments obtained after CNBr-cleavage (17) of the F3 dimer. Gel 1,2 and 3 correspond to peak I, II and III in fig. 3, respectively. All Gels were run for 3.5 hr except 2b and 3b for 1 hr.

trophoresis [7]. A similar situation pertains in chicken F3 histone. In an ether extract of acid hydrolyzed histone, acetic acid was identified on a LAC-446 diatoport S-column by gas chromatography. The acetyl content of F3 histone was found to be 0.35 mole per mole (corrected for losses by monitoring added radioactive acetate.)

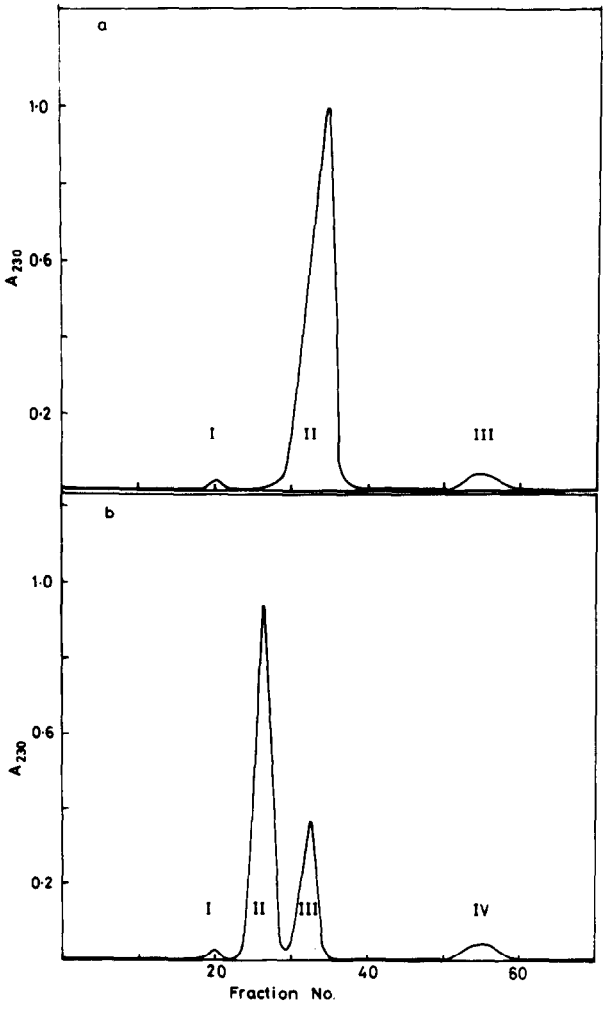


Fig. 2a. 'Sephadex G-100' column (15 × 100 cm) chromatography of crude chicken erythrocyte histone. Peak I corresponds to the outer volume and peak III to urea which was used to disaggregate the protein. Fig. 2b. 'Sephadex G-100' column chromatography of crude F3 chicken erythrocyte histone that had been oxidized with an equivalent amount of o-iodosobenzoate. Peak II corresponds to F3 dimer (fig. 1a, gel 4) and peak III corresponds to histone F2a2 which has the same mobility as F3 in this system. The eluant in both cases was 0.01 N HCl–0.02% sodium azide.

In chicken F3 histone, one cysteine residue per molecule was determined by spectrophotometric titration with *p*-chloromercuribenzoate [10] and a molecular weight of 17,000 estimated via the frictional ratio determined with the method of Parish et al. [11].

The amino acid analysis and the number of residues are given in table. From the amino acid composition the MW is calculated to be 15,500. Hydrazinolysis [12] yielded alanine as *C*-terminal amino acid. The *N*-terminal amino acid was identified as the DANSYL [13] and PTH-derivative [14] also as alanine.

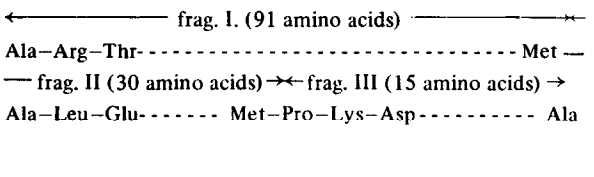
Table 1.
Amino-acid composition of F3 histone and CNBr-clea vage fragments.

	Histone F3	Fragment I	Fragment II	Fragment III	Sum I, II + III
Lys	8.58 (13)	10.85 (11)	3.93 (1)	6.57 (1)	13
Lys ε-N-meth	1.10	1.20	–	–	–
His	1.31 (2)	1.13 (1)	4.24 (1)	– (–)	2
Arg	13.48 (18)	14.62 (13)	3.73 (1)	25.81 (4)	18
Asp	3.81	2.37	6.89 (2)	6.51 (1)	5
Thr	7.52 (10)	9.07 (8)	6.54 (2)	0.02 (0)	10
Ser	4.42 (6)	5.54 (5)	3.77 (1)	0.03 (0)	6
Glu	11.87 (16)	11.24 (10)	14.46 (4)	14.31 (2)	16
Pro	4.62 (6)	5.75 (5)	– (–)	6.73 (1)	6
Gly	5.32 (7)	5.68 (5)	4.24 (1)	6.64 (1)	7
Ala	13.52 (18)	12.37 (11)	16.86 (5)	13.78 (2)	18
Cys ½	0.54 (1)	– (–)	2.25 (1)	– (–)	1
Val	4.41 (6)	4.47 (4)	6.21 (2)	0.02 (0)	6
Met*	1.34 (2)	0.51 (1)	2.77 (1)	– (–)	2
Ile	4.95 (7)	3.41 (3)	6.02 (2)	12.26 (2)	7
Leu	9.02 (12)	7.94 (7)	12.86 (4)	6.73 (1)	12
Tyr	2.19 (3)	2.21 (2)	3.16 (1)	– (–)	3
Phe	2.94 (4)	3.32 (3)	3.20 (1)	– (–)	4
NH_3	7.80	7.25	12.80	7.27 (1)	
Basic/ acidics B/A	1.57	2.08	0.5	1.66	
Residues/ molecule	136	91	30	15	136

* After CNBr-cleavage Met was analyzed as homoserine-lactone. Amino acids are expressed as mole percent of all amino acids recovered and residues per molecule (in parentheses). Hydrolyses were performed in 6 N HCl–0.2% phenol at 110° for 24 hr. Hydrolytic losses for Thr, Ser and Tyr were determined experimentally and corrections of 3%, 8% and 10% respectively made. Tryptophan was found to be absent as determined spectrophotometrically. Mono and dimethyl-ε-*N*-lysine were estimated together by the method of Gershey et al. [18] but using a shorter column.

F3 histone contains only two methionine residues and therefore lends itself ideally to CNBr-cleavage for the fragmentation of the molecule (fig. 3.). Fragment II contains the cystine residue, since on reduction with mercaptoethanol its molecular size becomes smaller as indicated by an increase of its retention volume on gel chromatography (graph not shown). In gel electrophoresis two of the fragments appear homogeneous, whereas the third shows the same microheterogeneity than the uncleaved F3 histone (fig. 1b, gel 1) indicating that this fragment contains the acetylated portion of F3. The amino acid composition of the fragments is given in table 1.

The absence of homoserine lactone in fragment III places it in the *C*-terminal position. The *N*-terminal sequences for three residues as determined by Edman degradations (14) in fragment I and the uncleaved histone are identical; namely: Ala–Arg–Thr–. Therefore fragment I has its position at the *N*-terminal and fragment II in the centre of the molecule. This arrangement together with the *N*-terminal sequences in fragment II and III allows to assign to F3 histone from chicken erythrocyte the following tentative structure:



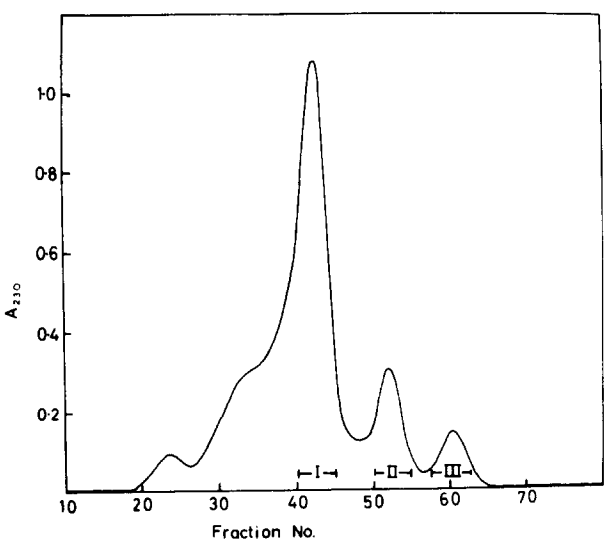


Fig. 3. 'Sephadex G-100' column (1.5 × 100 cm) chromatography of the CNBr cleavage products of the F3 dimer. The dimer was dissolved in 70% formic acid and cleaved with a 100-fold excess of CNBr at room temperature for 24 hr [17]. The other peaks and shoulders preceding I are due to unreacted and partially cleaved histone. The eluant was 0.01 N HCl–0.02% sodium azide.

Like other histones F3 also shows a clustering of basic amino acids. The *C*-terminal region has a basic to acidic amino acid ratio of 1.66 while the cysteine residue is situated in a very acidic region (B/A 0.5). The large *N*-terminal fragment is very basic (B/A:2.0), and contains all the acetylated and methylated lysine residues (table 1).

Recently DeLange et al. [15] published the amino acid sequence of a *C*-terminal fragment of calf thymus histone F3. It is interesting to note that the amino acid composition of our fragment III and its *N*-terminal sequence of Pro–Lys–Asp are identical to the corresponding region in calf thymus F3 histone. Whereas this may indicate a close similarity between F3 histones from calf thymus and chicken erythrocytes, with respect to their cysteine content, the two histones differ markedly. Chicken F3 on oxidation due to the presence of only 1 cysteine forms a dimer with decreased electrophoretic mobility. The corresponding histone from calf thymus after treatment with *o*-iodosobenzoate, results predominantly in a compound with increased electrophoretic mobility identical to that of histone F2b. This may indicate that calf thymus F3 contains more than one cysteine sterically arranged in such a way that on oxidation an internal disulphide bond is established resulting in a conformational change of the molecule with increased electrophoretic mobility in polyacrylamide gel.

Acknowledgements

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