THE OCCURRENCE OF HISTONE H3 AND H4 IN YEAST

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1. Introduction

Histones occur in a wide variety of uni- and multicellular organisms. Histone H4 is evolutionarily a very stable protein [1]. We have shown a similar conservation of the primary structure for histone H3 isolated from chicken [2], shark [3], sea urchin, a mollusc and a plant [4]. The other 3 histones H1, H2A and H2B seem to show a greater variability in their structures [5]. Whereas for multicellular organisms considerable information on the primary structures of histones is available to allow general conclusions to be drawn as to their occurrence, their identity and general evolutionary trends of their structures, this does not apply to unicellular, eucaryotic organisms. This lack of information is probably related to the difficulties experienced in the purification of the small amounts of histones present in the chromatin of these organisms the genome of which is by several orders of magnitude smaller than that of higher organisms. The occurrence of histones and their identity in fungi, green algae and protozoa is controversial [6,7]. In yeast the presence of histones H2A, H2B and H4 has been reported [8], whereas it has been found that histones H1 and H3 were absent [8].

If histones were to be essential for the structural and functional organisation of chromatin the absence from yeast of the conservative histone H3 would cast doubt on such a view. We have therefore reinvestigated the question of the occurrence and the nature of histones in yeast.

2. Materials and methods

Chromatin was isolated from pressed bakers yeast essentially by the method of Tonino and Rozijn [9]. The crude nucleoprotein was spun through 1 M sucrose in a SS-34 rotor (Sorvall) at 20 000 rev/min for 2 h. The resulting pellet was washed twice each with 0.05 M Tris, 1 mM $MgCl_2$, pH 8 buffer; 0.05 Tris, pH 8, buffer and with saline citrate (0.14 M NaCl–0.01 Na citrate) followed by one wash with 0.05 M citric acid. Histones were then extracted with 0.25 M HCl followed by a precipitation with 8 volumes of acetone in the presence of approximately 0.2 N H_2SO_4 [10].

Analytical polyacrylamide electrophoresis was performed essentially by the method of Panyim and Chalkley [11]. Amino acid analysis and sequential degradation were performed as described before [2]. Amino acid analysis on protein fractions separated on polyacrylamide gels using ethylene diacrylate as cross-linker was done similar to Jockusch and Walker [12]. Briefly polyacrylamide gels were made from the same solution as the analytical gels [11] except that they contained 8% acrylamide and 0.2% ethylene diacrylate. Approx. 100–120 μg crude histone fraction was applied per gel. Electrophoresis was performed for 1.5 h. The gel slices containing histone H3 and H4 were solubilized in a solution of 0.1 N NaOH–2% Na_2CO_3 and precipitated from the solubilized gel by the addition of 100% (w/v) trichloroacetic acid to a final concentration of 30% (w/v). Histones were recovered by centrifugation at 12 000 g for 20 min. The trichloroacetates of histones were then washed in distilled H_2O. Further experimental details are given in the legends to figures and tables.

3. Results and discussion

On polyacrylamide electrophoresis the 0.25 N HCl extract of yeast chromatin reveals the presence of 4–6

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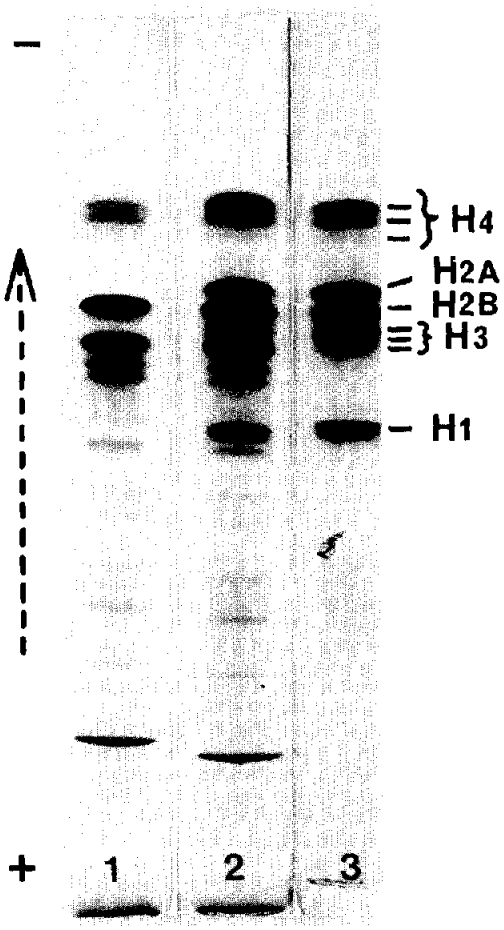
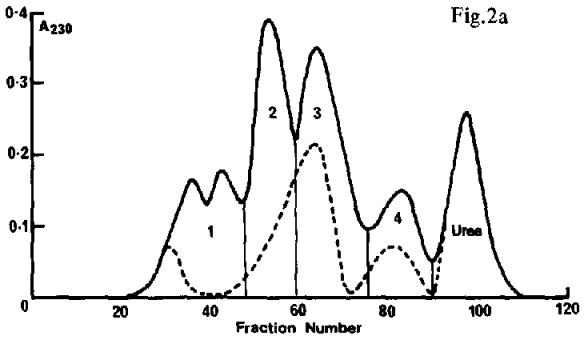


Fig.1. Electrophoretic pattern of total yeast histones (gel 1), total calf thymus histones (gel 3) and of a mixture of calf thymus and yeast histones (gel 2).

predominant fractions with mobilities in the region of calf thymus histones (fig.1). The fastest moving fraction has an identical mobility to histone H4 and shows the same subfractions of the latter probably also due to fractional acetylation [13]. The electrophoretic mobility of the following fraction is identical to that of calf histone H2B. The remainder of the prominent fractions have mobilities slower than calf thymus histone H3. In particular there are no fractions present in this yeast chromatin preparation with mobilities of calf histones H3 and H1.

This crude histone preparation, still considerably contaminated with slower moving proteins, is usually isolated in amounts of approx. 20 mg per 100 g pressed yeast. On gel filtration this crude histone can be resolved into 4 fractions (fig.2a and 2b) which are still contaminated with a large number of different proteins in small concentration. They become evident after restaining the amido black stained gels with Coomassie blue, a more sensitive protein dye (fig.2). This explains



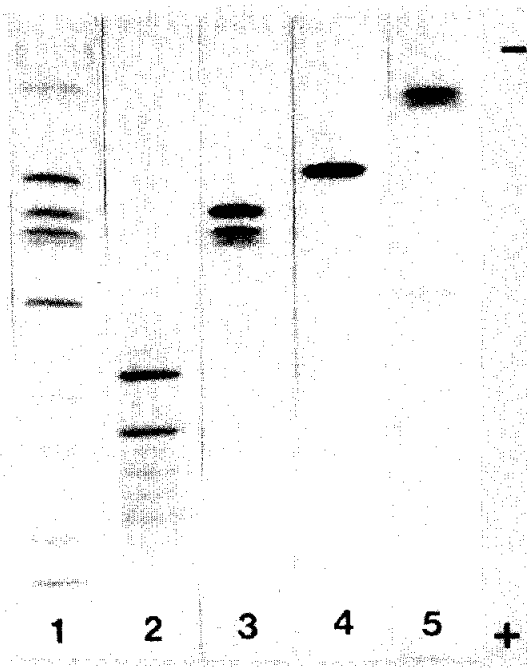


Fig.2(a). Elution profile of total yeast (——) and calf thymus histones (– – – –). 10 mg yeast dissolved in 6 M urea–1% mercaptoethanol were applied to a 1.6 × 100 cm Biogel P-60 column and eluted with 0.01 N HCl, 1.5 ml fraction volume: 1.5 ml. (b) Electrophoretic pattern of the molecular exclusion chromatography fractions. Fractions were pooled as indicated (fig.2a). Gel 1 corresponds to total yeast histones and gel 2 to 5 to fraction 1 to 4.

Table 1
Amino acid composition[a] of yeast- and plant histones

	Column fraction no.			Electrophoretically purified fraction		Pea	Electrophoretically purified fraction		Pea
	2	3	4		3	H3 [15]		4	H4 [1]
	mole %	mole %	mole %	mole %	residues/mole[b]	residues/mole[b]	mole %	residues/mole[b]	residues/mole[b]
Asp	7.1	6.7	6.8	4.1	5.5 (6)	5	4.9	5.0 (5)	5
Thr	6.3	5.8	4.9	5.7	7.7 (8)	10	5.6	5.7 (6)	7
Ser	8.7	6.8	6.1	6.2	8.3 (8)	6	5.9	6.0 (6)	2
Glu	10.6	13.2	9.9	11.6	15.6 (16)	15	7.7	7.8 (8)	6
Pro	6.5	4.4	2.7	4.5	6.1 (6)	6	1.2	1.2 (1)	1
Gly	6.6	6.0	11.0	5.4	7.3 (7)	7	13.9	14.2 (14)	17
Ala	11.9	10.2	7.1	11.1	15.1 (15)	19	6.7	6.8 (7)	7
½Cys	n.d.	0.3[c]	n.d.	n.d.	(0)	1	n.d.		0
Val	4.7	4.9	6.0	5.3	7.2 (7)	6	7.1	7.2 (7)	8
Met	0.7	0.5	0.5	0.0	0 (0)	1	0.1	0.1	1
Ile	5.0	4.8	5.5	4.3	5.8 (6)	7	5.3	5.4 (5)	7
Leu	7.4	8.6	7.7	9.6	13.0 (12)	12	8.0	8.2 (8)	8
Tyr	2.9	1.9	3.9	1.6	2.1 (2)	2	3.9	3.9 (4)	4
Phe	1.7	3.2	2.2	4.5	6.1 (6)	5	2.4	2.4 (2)	2
Lys	12.5	10.9	12.6	11.3	15.2 (15)	14	11.4	11.6 (12)	10
His	2.2	1.8	2.2	1.8	2.4 (2)	2	2.0	2.0 (2)	2
Arg	5.2	10.1	10.7	13.2	17.8 (18)	17	14.1	14.3 (14)	15
B/A	1.12	1.14	1.53	1.67	1.65		2.18		2.45
Lys/Arg	2.50	1.08	1.18	0.85	0.82		0.81		0.67
N-terminal amino acid	Blocked	Ala	Blocked						

[a] No corrections have been made for destruction of amino acids or incomplete hydrolysis;
[b] Calculated for 135 amino acids in H3 and 102 amino acids in H4;
[c] Determined as cystic acid [16];
n.d. = not determined.

why the amino acid composition of column fraction 3 and 4 do not match those of any of the known histones. A similar composition of yeast histone like proteins has been reported previously from another laboratory [8]. However, after further purification through preparative gel electrophoresis fraction 4 resembles more closely calf thymus histone H4 and fraction 3 calf histone H3 (table 1). In particular in both proteins the Lys/Arg ratio and the ratio of basic amino acids/acidic amino acids approach more closely the corresponding ratio in the histones from higher organisms. In both yeast histones, H3 and H4, methionine and cystein appear to be absent. This is consistent with the observation that on treatment with CNBr neither of the yeast histones yields fragments nor does the H3 histone give on oxidation with iodosobenzoate a dimer as histone H3 from other sources does due to its cystein content [2].

A partially purified preparation of fraction 3 was subjected to sequential N-terminal degradation. At each degradation cycle a background of many amino acids became obvious indicating the presence of a considerable number of low concentration contaminants. However, against this background in each cycle a predominant amino acid could be identified unequivocally (table 2). Over the 15 degradation cycles performed the N-terminal sequence of the major protein component in fraction 3 proved to be identical to that of histone H3 isolated from sources evolutionarily widely separated from each other [4]. As in histone H3 isolated from plants, the lysine residue 4 in yeast H3 is methylated.

Table 2
Sequential degradation of fraction 3

Degradation step	Assigned amino acid	Yield (nmoles) of assigned amino acids[a] R−1	R	R+1
1	Ala	–	63	15
2	Arg	8	29	7
3	Thr	11	42	14
4	Lys[b]	10	29	10
5	Gln[c]	11	37	13
6	Thr	14	34	14
7	Ala	8	43	17
8	Arg	5	20	8
9	Lys	11	19	12
10	Ser	4	12	4
11	Thr	11	21	13
12	Gly	8	22	22
13	Gly	22	22	13
14	Lys	6	15	5
15	Ala	12	27	13
16				

[a] R: yield of assigned amino acid together with its yield at the preceding (R−1) and following (R + 1) cycle
[b] includes approximately 50% methylated ε-*N*-Lys
[c] sum of PTH-Glu and PTH-Gln

Yields of some of the amino acids recovered after each cycle of the automatic sequential Edman degradation of fraction 3 (fig.2a). Amino acids have been quantitated as the PTH-derivatives by gas chromatography except for Arg and Lys-derivatives which have been determined after rehydrolysis to the amino acids [2]. No corrections for destruction have been made, which in the case of Arg and Lys amount to approx. 50%.

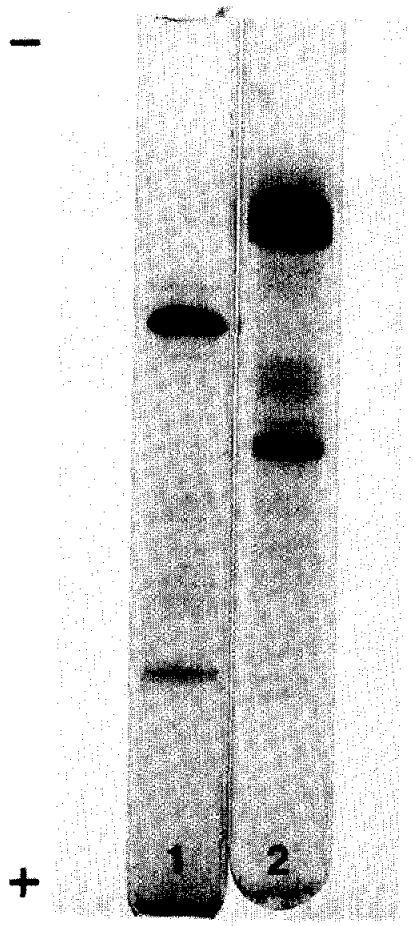


Fig. 3. Electrophoretic pattern of column fraction 3 and fraction 4 (fig.2a) stained with amido black first (fig2b) followed by Coomassie blue. Gel 1 = fraction 3; Gel 2 = fraction 4.

At this site methylation does not occur in histones of animal origin [4].

These investigations show that from yeast chromatin a histone can be isolated which is identical in its electrophoretic mobility to the histone H4 from higher organisms. This yeast histone has a blocked N-terminus as has histone H4. Its amino acid composition is very similar to that of histone H4 as is the elution volume from a molecular sieve column. A second histone can be isolated from yeast chromatin which in its amino acid composition and elution volume is very similar to histone H3 from other sources though it differs in its electrophoretic mobility. The N-terminal sequence of the first 15 amino acids is identical to that of histone H3. In addition, yeast contains other histone-like proteins with electrophoretic mobilities between calf histone H3 and H1. The former are similar in electrophoretic mobility and amino acid composition (fig.4 and table 1) to those described previously to occur in plants [14]. In this investigation no proof has been found for the presence of histones resembling the type H2A or H2B.

The presence of histone H3 and H4 in yeast supports the view that these two highly conserved histones are essential for the structure of chromatin.

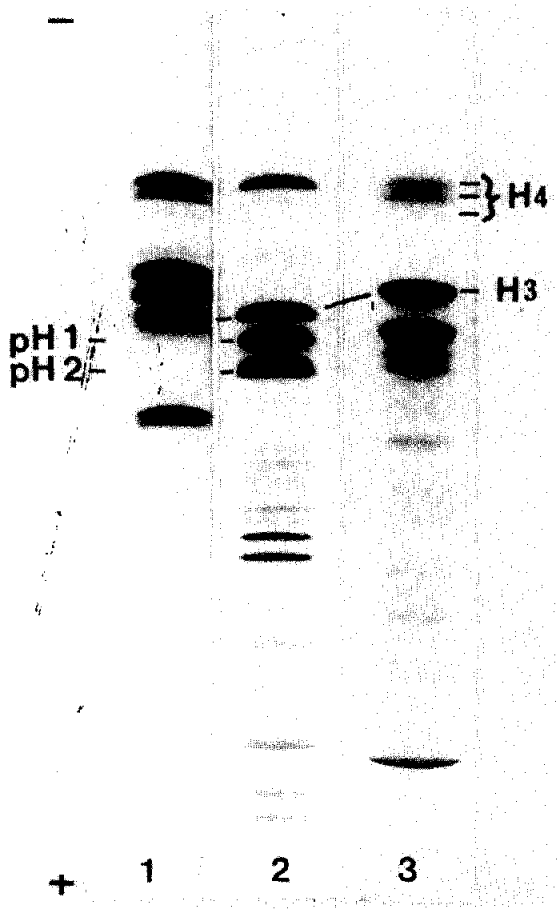


Fig.4. Comparison of the electrophoretic pattern of total histones from calf thymus (gel 1) cycad pollen (gel 2) and yeast (gel 3). PH1 and PH2 typical plant histones (14).

Acknowledgements

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