ISOLATION AND CHARACTERIZATION OF THE HISTONES FROM CYCAD POLLEN

W. F. BRANDT and C. VON HOLT

Department of Biochemistry, University of Cape Town, South Africa

Received 12 December 1974

1. Introduction

Sequence studies of calf and pea histone F3 and F2a1 have revealed that these histones are evolutionarily very stable proteins [1,2]. It is desirable to obtain additional comparative information on histones in general. Such investigations in plants are hampered by the low nucleoprotein concentration of plant cells and the difficulty of separating the nucleoprotein complex of vegetative tissue from the non-chromatin components. Using pollen as a source of nucleoprotein should overcome some of these difficulties.

The male cones of the gymnosperm *Encephalartos caffer* (cycad) yielded large amounts of pollen from which we isolated nucleoprotein in high yields.

We wish to report the isolation of this cycad nucleoprotein and subfractionation of its histones into various groups according to their solubility and behaviour on gel exclusion chromatography. The partial amino acid sequence of the histone F3 isolated from this source has been published in a previous communication [3].

2. Materials and methods

Cycad pollen (*Encephalartos caffer*) were collected by shaking ripe male cones in plastic bags and subsequently sieving the crude pollen through a small gauge nylon mesh. All subsequent operations were performed between 2–4°C. The pollen grains were suspended in 0.14 M NaCl – 0.01 sodium citrate – 0.01 M $Na_2S_2O_5$ – 0.01 M EDTA and ruptured in a cell homogenizer (model MSK B. Braun [4]), set to full speed for 2 min using 0.5 mm glass balls. The homogenate was subjected to centrifugation at 30 000 *g* for 10 min. The precipitate, which consisted mainly of ruptured pollen envelopes and undissolved nucleoprotein, was washed 6 times with the buffer medium by repeated homogenization with a Dounce homogenizer, followed by centrifugation at 10 000 *g* 10 min. The nucleoprotein from the pellet was finally extracted with 2 M NaCl followed by centrifugation at 40 000 *g* for 20 min. The clear and viscous supernatant was slowly poured into cold distilled H_2O (final NaCl conc. 0.2 M) and the nucleoprotein removed by winding it around a glass rod (purified chromatin).

Alternatively, crude nucleoprotein was isolated by removing the ruptured and empty pollen grains by centrifugation at 500 *g* for 5 min. Approx. two thirds of the nucleoprotein remained in the supernatant and was recovered by centrifugation at 30 000 *g* for 10 min. The sticky pellet was washed with the wash medium as described, leaving undissolved crude nucleoprotein.

The DNA, RNA and total proteins in the purified nucleoprotein were determined colorimetrically as described by Paoletti and Huang [5]. Histones were estimated after extraction with 0.4 H_2SO_4 [5].

Total histones were extracted from the nucleoprotein on a preparative scale with 0.25 M HCl and subsequently recovered by the addition of 5 vol of cold acetone [6]. Histone fractions were also isolated by selective extraction of nucleoprotein with ethanolic HCl according to the method 2 of Johns [7]. Histones were hydrolyzed in constant boiling HCl – 1% thioglycollic acid for 24 hr [8]. Electrophoresis was carried out essentially by the method of Panyim and Chalkley [9]. Endgroups of histones were determined by a single Edman degradation [8].

North-Holland Publishing Company – Amsterdam

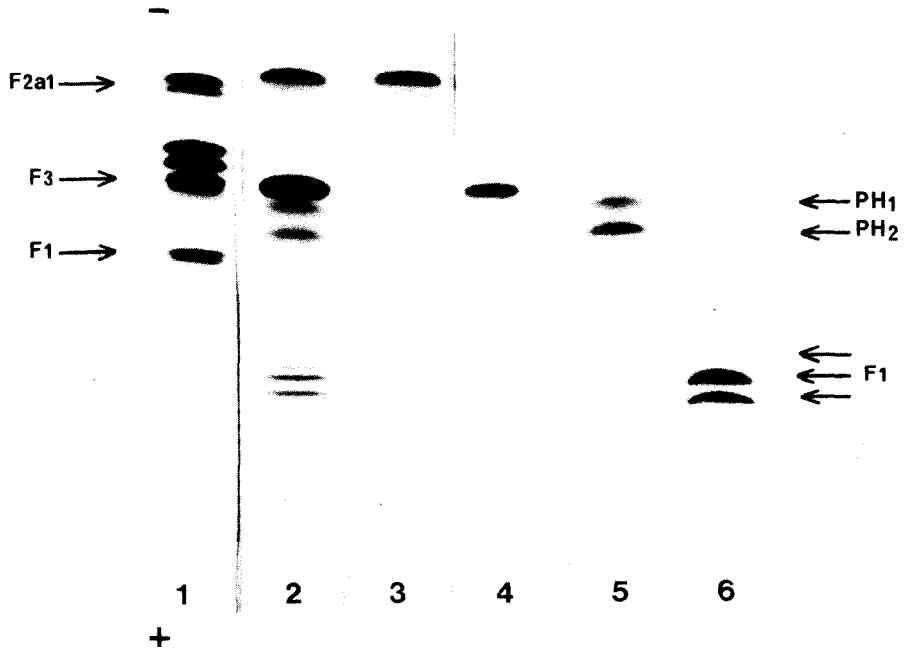

Fig.1. Gel electrophoretic pattern of cycad histones. Gel 1 corresponds to total calf thymus histones; 2: total cycad pollen histones; 3: cycad histone F2al; 4: cycad histone F3; 5: cycad histone PH1 & PH2 [11]: and 6: cycad F1 histones. All samples were dissolved in 6 M urea - 1% mercaptoethanol.

3. Results and discussion

In contrast to other plant tissues [3] cycad pollen was found to yield relatively large amounts of nucleoprotein. In a typical isolation 20 g of dry pollen yielded 900 mg of freeze-dried nucleoprotein after the 2 M NaCl extraction step. This nucleoprotein readily dissolved in distilled water forming a viscous gel. The absorption spectrum of this nucleoprotein in 2 M NaCl is very similar to that isolated from peas [3]. The ratio of the A_{260} nm to A_{245} nm was found to be 0.57. The chemical composition of this cycad nucleoprotein was determined [5] and the ratio of DNA: histones: non-histones: RNA was found to be 1:0.95:0.38:0.07. In comparison, calf thymus nucleoprotein isolated by the method of Mauritzen et al. [10] yielded by the same analytical procedures the following ratios: 1:1.13:0.23:0.04.

The electrophoretic properties of total histones, extracted with 0.25 N HCl, are compared to those of the corresponding fraction from calf thymus in fig.1. From the electrophoretic pattern it is apparent that the cycad pollen contains only two histone fractions which correspond closely in their mobility to calf thymus histones, namely F2al and F3. Protein with mobilities identical to calf thymus F2a2 and F2b, as well as to F1, are absent in cycad. However, two proteins with slower electrophoretic mobility than F3 and calf thymus F1 respectively are present.

The identities of the F2al and F3 fractions were established by obtaining pure F2al on exclusion chro-

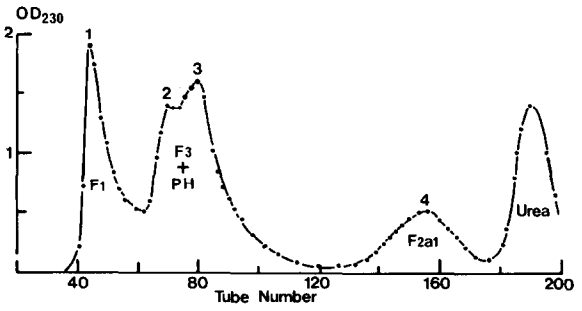

Fig.2. Elution pattern of cycas total histones on Biogel P-60 (column 2.5 × 150 cm) using 0.05 M NaCl 0.02 N HCl as eluent. The fraction volume was 4.5 ml. The protein (65 mg) was dissolved in urea-mercaptoethanol. Polyacrylamide gels have been run and are shown in fig.1.

Table 1
Amino acid composition of cycad pollen histones

	F2al cycad	F2al* pea	F3 cycad	F3* pea	$PH_{1,2}$ cycad	'F1' cycad
	mol/100 mol					
Lys	10.3	9.8	9.1	10.4	18.8	22.4
ε-*N*-MeLys	T	–	1.3	ND	ND	ND
His	2.2	2.0	1.7	1.5	2.0	0.8
NH_3	6.3	4.9	9.2	6.7	14.1	9.3
Arg	14.8	14.7	13.4	12.6	4.4	3.1
Asp	4.7	4.9	3.8	3.7	4.5	3.7
Thr	5.7	6.9	6.5	7.4	5.0	5.6
Ser	1.7	2.0	3.2	4.4	6.4	6.7
Glu	6.8	5.9	11.3	11.1	10.9	7.2
Pro	1.9	1.0	4.6	4.4	7.5	10.3
Gly	16.0	16.7	5.6	5.2	6.7	4.2
Ala	7.3	6.9	14.5	14.1	11.3	22.4
Cys½	–	–	0.6	0.7	ND	ND
Val	7.3	7.8	4.7	4.4	6.4	3.4
Met	1.0	1.0	0.7	0.7	1.1	0.4
Ile	6.5	6.9	4.7	5.2	5.4	2.3
Leu	7.9	7.8	8.9	8.9	5.1	4.0
Tyr	3.8	3.9	1.6	1.5	2.0	1.7
Phe	2.1	2.0	3.8	3.7	2.5	1.3
N-terminal	blocked	blocked	Ala	Ala	blocked	blocked

ND – not determined
* – results have been calculated from the amino acid sequence [14,20] Experimental values represent duplicates of 24 hr hydrolysates. No corrections for incomplete hydrolysis and destruction were applied. The extent of destruction of Thr and Ser are about 5% and 10% respectively.

matography on Biogel P-60 (fig.2). The histone F3 isolated via exclusion chromatography was still contaminated with two proteins, probably identical [11] to the so-called plant histones (PH). This 'F3' fraction or a crude histone F3 preparation obtained by selective extraction with ethanol–HCl [7] could readily be purified by dimerisation of F3 histone followed by chromatography on Sephadex G-100 [12].

The amino acid composition of the cycad histones F2al and F3 compared in table 1 to the corresponding fractions from pea, reveals that they are very closely related. Histone F3 subjected to 48 Edman degradations [13] was found in its N-terminal sequence to be identical to pea histone F3 [14]. It differs from animal histone F3 in that Tyr-41 is replaced by Phe [13] and in the degree of acetylation and methylation of lysine residues. Histone F2al, like in other organisms [5], has a blocked N-terminus and has not yet been subjected to sequence studies. The two proteins (PH), having a lower electrophoretic mobility than F3 (fig.1) have been obtained in a partially purified form by Johns' selective extraction (method 2 [7]). They were found to behave in this extraction like fraction F2b. In contrast to histone F2b from calf thymus, however, these two histones were not precipitated by 0.5 M perchloric acid [7]. Gel filtration chromatography [16] or chromatography on amberlite [17] failed to separate the two fractions (PH_1 and PH_2) indicating that they have closely related properties.

The amino acid composition of the mixture of these two plant histones resembles the composition of the histone from calf fraction IIa and IIb isolated from pea plants by Bonner et al. [17], pea histone F2a2 of Sommer and Chalkley [18], band 4 and 5 (type F2b) of Autran and Bourdet [19], and has some similarities to calf thymus histone F2b [17]. Both PH

histones from cycad are blocked. A protein fraction behaving like histone F1 on exclusion chromatography (fig.2) was also isolated. The electrophoretic pattern of this fraction is shown in fig.1, gel 6, and it is evident that the electrophoretic mobility is substantially less when compared to calf thymus F1, and that this fraction is heterogeneous. The amino acid composition resembles that of the plant histone (pea F1) and calf histone F1. This fraction is extremely susceptible to proteolytic degradation even at temperatures below 0°C as is cycad histone F3.

Our results show that the investigated gymnosperm *Encephalartos caffer* possesses a histone complement which is very similar to that found in angiosperm plants [11,17,19]. Despite a considerable amount of effort especially using amberlite column chromatography [17], no F2a2 type of histone could be isolated. This is contrary to the findings of Autran and Bourdet [19] which showed that the electrophoretic pattern of wheat histone is closely related to cycad histone and isolated a F2a2 type histone, but no F3 histone. Histone F2a1 and F3 in cycad by electrophoretic and chromatographic criteria and their amino acid composition are very closely related to the corresponding animal histones. The evolutionary stability of histone F3 is borne out by the identity of the previously published N-terminal amino acid sequence [13] of that histone isolated from cycad pollen. In addition, plants characteristically seem to contain two groups of histones, namely the so-called plant histones (PH) and an electrophoretically slow moving histone group which cannot obviously be related to histones F1 in animals. Although the amino acid composition relates these two groups of histones vaguely to histone F2b and F1 present in higher organisms [17], sequence studies would be required to establish such a relationship.

Acknowledgements

We thank the C.S.I.R. (R.S.A.) and the University of Cape Town Research Committee for financial support.

References

[1] Hnilica, L. S. (1972) in: The Structure and Biological Functions of Histones (L. S. Hnilica, ed.) The Chemical Rubber Co.
[2] Patthy, L., Smith, E. L. and Johnson, J. (1973) J. Biol. Chem. 248, 6834.
[3] Brandt, W. F., Strickland, W. N., Morgan, M. and Von Holt, C. (1974) FEBS Lett. 40, 167.
[4] Merkenschlager, M., Schlossmann, K. and Kurz, W. (1957) Biochemische Zeitschrift 329, 332.
[5] Paoletti, R. A. and Huang, R. C. C. (1969) Biochem. 8, 1615.
[6] Johns, E. W. (1971) in: Histones and Nucleohistones (D. M. P. Phillips, ed.) Plenum Press, London, p. 10.
[7] Johns, E. W., Phillips, D. M. P., Simson, P. and Butler, J. A. V. (1960) Biochem. J. 77, 631.
[8] Brandt, W. F. and Von Holt, C. (1974) Eur. J. Biochem. 46, 407.
[9] Panyim, S. and Chalkley, R. (1969) Arch. Biochem. Biophys. 130, 337.
[10] Mauritzen, C. M., Starbuck, W. C., Saroja, I. S., Taylor, C. W. and Busch, H. (1967) J. Biol. Chem. 242, 2240.
[11] Nadeau, P., Pallotta, D. and Lafontaine, J. G. (1974) Arch. Biochem. Biophys. 161, 171.
[12] Brandt, W. F. and Von Holt, C. (1971) FEBS Lett. 14, 338.
[13] Brandt, W. F., Strickland, W. N. Morgan, M. and Von Holt, C. (1974) FEBS Lett. 40, 167.
[14] DeLange, R. J., Hooper, J. A. and Smith, E. L. (1972) Proc. Nat. Acad. Sci. U.S. 69, 882.
[15] DeLange, R. J., Fambrough, D. M., Smith, E. L. and Bonner, J. (1969) J. Biol. Chem. 244, 5669.
[16] Böhm, E. L., Strickland, W. N., Thwaits, B. H., Van der Westhuyzen, D. R. and Von Holt, C. (1973) FEBS Lett. 34, 217.
[17] Fambrough, D. M. and Bonner, J. (1966) Biochem. 5, 2563.
[18] Sommer, K. R. and Chalkley, R. (1974) Biochem. 13, 1022.
[19] Autran, J. C. and Bourdet, A. (1973) Comptes rendus 277, Série D, 2553.
[20] DeLange, R. J., Fambrough, D. M., Smith, E. L. and Bonner, J. (1969) J. Biol. Chem. 244, 5669.