Volume 191, number 1 FEBS 3001 October 1985

On the ubiquitous presence of histone acetyltransferase B in eukaryotes

M. Luisa Salvador, Ramón Sendra, Gerardo López-Rodas, Vicente Tordera and Luis Franco

Department of Biochemistry, Faculties of Sciences, University of Valencia, Burjassot, Valencia, Spain

Received 19 July 1985

Histone acetyltransferase B activity has been found in pea (*Pisum sativum*) seedlings. The enzyme has been partially purified and it has been found that it is highly specific for H4. The results confirm that histone acetyltransferase B occurs in 3 eukaryotic kingdoms.

Histone acetyltransferase B Pisum sativum *Chromatin* *Histone acetylation*

1. INTRODUCTION

Since the original suggestion by Allfrey and his co-workers [1] that the acetylation of histones may play a role in the control of transcription, much research on this modification of histones has been carried out, and some recent revisions have appeared [2–4]. As McCarty et al. [5] pointed out, the understanding of the role of histone acetylation will require detailed information on the enzymes involved in this process. Histone acetyltransferases fall, in most cases, into 2 categories: class A, nuclear enzymes, and class B, present in the cytoplasm [5,6]. While the former may be involved in the regulation of gene expression, the latter are thought to be necessary for histone acetylation prior to chromatin assembly [7]. The study of plant acetyltransferases, which had not been undertaken to date, seemed to be interesting. Plant histones are acetylated to a very small extent [8–10], but a canonical acetyltranferase B must be present if the above-mentioned hypothesis on its role proves to be correct. This work reports the partial purification and some properties of a putative histone acetyltransferase B from pea seedlings.

2. MATERIALS AND METHODS

Protamine sulphate, poly(L-lysine) and bovine serum albumin were purchased from Sigma, and [1-^{14}C]acetylcoenzyme A (57.2 Ci/mol) and Amplify were obtained from Amersham. All other reagents were of analytical grade. Peas (*Pisum sativum*, var. Lincoln) were germinated and grown in the dark at 27°C.

5-day-old pea seedlings were used in the following experiments. Cotyledons were excised and the plant material ground in a mortar with acid-washed sand and 1 vol. buffer A (1 mM $MgCl_2$, 0.25 mM EDTA, 10 mM β-mercaptoethanol, 50 mM $NaHSO_3$, 0.7 mM phenylmethylsulfonyl fluoride and 75 mM Tris-HCl, pH 7.9). The homogenate was filtered through Miracloth and the retained sand and debris were washed 3 times with 1 vol. buffer A. The combined filtrates were centrifuged at 1200 × *g* for 10 min and solid $(NH_4)_2SO_4$ was added to the supernatant to obtain a saturation of 25%. After standing at 4°C for 30 min, solid $(NH_4)_2SO_4$ was added to obtain a 90% saturation. The solution was stirred at 4°C for 2 h and the precipitate was recovered by centrifugation at 30000 × *g* for 20 min, dissolved in buffer B (0.25 mM EDTA, 10 mM NH_4Cl, 5 mM β-mercaptoethanol, 20% (v/v) glycerol, 15 mM Tris-HCl, pH 7.9), and dialyzed against 3 changes

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

of buffer B. The clarified dialyzate was used as a source of histone acetyltransferases, which were purified essentially as described for yeast acetyltransferases [6], with the exceptions given in section 3.

Chicken erythrocyte nuclei were obtained as described by Weintraub et al. [11] and stored at −25°C in the presence of 66% glycerol until use. Pea seedling nuclei were prepared by a modification of the method of Spiker et al. [12] that will be described elsewhere (Ull, M.A. and Franco, L., in preparation). Histones were obtained by acid extraction of nuclei. All other methods were carried out as described [6].

3. RESULTS

The first step in the histone acetyltransferase purification was chromatography on DEAE-Sepharose, developed with a gradient (10–350 mM) of NH_4Cl. The elution profile is shown in fig.1.

The histone acetyltransferase activity was recovered as a single peak, which elutes at 300 mM NH_4Cl. Due to the high efficiency of non-catalytic transfer of acetate to histone acceptors when the concentration of NH_4Cl is below 100 mM [6], only the activity of fractions eluting at gradient concentration higher than that value is represented in fig.1. The tailing of non-enzymatic peak, which extends in this experiment to almost 200 mM NH_4Cl, makes it difficult to decide whether a true histone acetyltransferase activity is also present in these fractions.

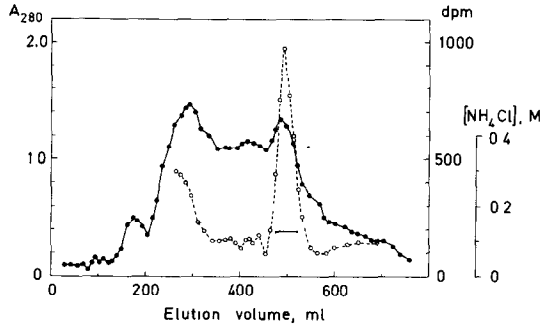

Fig.1. Elution of histone acetyltransferase from DEAE-Sepharose. Results from an experiment with 115 g pea seedlings. (●) A_{280}, (○) histone acetyltransferase activity, (···) NH_4Cl gradient.

The fractions showing histone acetyltransferase activity, marked with a bar in fig.1, were pooled, precipitated with solid $(NH_4)_2SO_4$ to 85% saturation, redissolved in buffer B, and dialyzed against the same buffer, except that the concentration of NH_4Cl was 100 mM. The solution was centrifuged at 12000 × *g* for 30 min and chromatographed in an Ultrogel AcA34 column (fig.2). The histone acetyltransferase activity eluted as a single peak, corresponding to M_r 160000, which was used as enzymatic extract for the experiments described below.

The recovered enzymatic activity actually corresponds to a histone acetyltransferase. It does not catalyze, at all, the transfer of acetate to poly(L-lysine) or protamine, and bovine serum albumin is acetylated to an almost negligible extent (only 3% of the activity incorporated into histones). Both chicken and pea histones are good substrates of the enzyme. Electrophoretic separation of histones after the acetylation reaction revealed that acetate is exclusively incorporated into H4 (fig.3). This striking specificity for H4 was equally manifest when chicken histones were used as substrates (fig.3).

We have also tested the temperature and pH dependence of pea histone acetyltransferase. In both instances, parallel blanks with heat-denatured

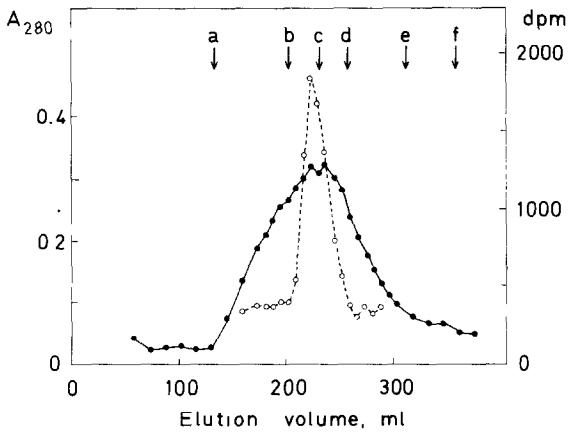

Fig.2. Purification of pea seedling histone acetyltransferase on Ultrogel AcA34. (●) A_{280}, (○) acetyltransferase activity. Arrows, elution volume of M_r markers: (a) dextran blue, M_r 2000000; (b) beef liver catalase, M_r 240000; (c) rabbit muscle aldolase, M_r 158000; (d) yeast invertase, M_r 120000; (e) egg yolk ovalbumin, M_r 45000; (f) egg lysozyme, M_r 14000.

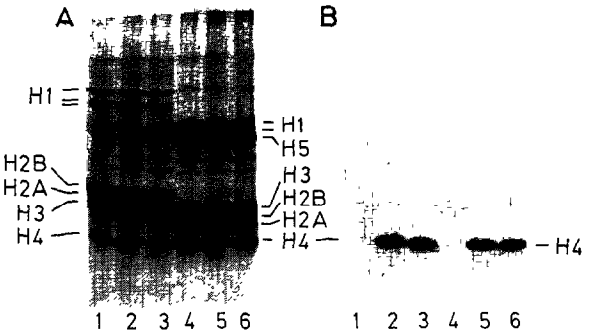

Fig.3. Specificity of histone acetylation by pea seedling histone acetyltranferase. After incubation with [1-^{14}C]acetylcoenzyme A, histones were separated by SDS-polyacrylamide gel electrophoresis. (A) Coomassie-stained gel, (B) fluorogram. Lanes: 1–3, pea histones; 4–6, chicken histones. In lanes 1 and 4 the enzymatic extract was substituted for 100 μl of 110 mM NH_4Cl to show that, under the conditions of the assay, non-enzymatic transfer of acetate to histones is negligible. Lanes 2–3 and 5–6 are duplicate incubations with slightly different histone amounts.

enzyme were run, because non-enzymatic transfer of acetate from acetylcoenzyme A to histones becomes prominent at high temperature as well as at high pH values. Once the blank values were subtracted, it was found that the enzyme had a broad pH optimum between 7.9 and 8.6 and a sharp optimum temperature around 37°C. At this temperature, the enzymatic incorporation of acetate into histones is linear for at least 25 min, showing that the degradation of the enzyme is negligible under the experimental conditions used to assay the enzymatic activity.

Finally, some experiments in which nuclear fractions from pea seedlings were used as a source of histone acetyltransferase failed to reveal any measurable enzymatic activity. This may be taken as an indication of the cytoplasmic origin of the enzyme described.

4. DISCUSSION

Our results strongly suggest that the histone acetyltransferase described corresponds to the cytoplasmic or B form found in other organisms [5,6,13–16] because of its apparent absence from nuclei, its preference for H4 and, also, due to its late elution in NH_4Cl gradients. Nuclear acetyltransferase usually eluted at lower ionic strength values (equivalent to about 150 mM NH_4Cl). Our results do not exclude the presence of some other histone acetyltransferase in pea seedlings, but if an A enzyme is present in pea seedling nuclei, its activity has to be very low.

The specificity of the reported enzyme for H4 is remarkable. Proteolytic activity is virtually absent from the enzyme preparation as revealed by the low level of peptides running faster than the histones in fig.3. This assures that the exclusive use of H4 as substrate is not an artifact due to the proteolytic degradation of other potential histone substrate. A similar exclusive specificity for H4 has been reported for a *Drosophila* acetyltransferase B [17].

The present data and our recent report of a histone acetyltransferase B in yeast [6] confirm the existence of acetyltransferase B in 3 eukaryotic kingdoms. This ubiquitous presence may be related to the role proposed in the assembly of chromatin.

ACNOWLEDGEMENT

This work has been supported by grant 1101/81 from CAICYT.

REFERENCES

[1] Allfrey, V.G., Faulkner, R.M. and Mirsky, A.E. (1964) Proc. Natl. Acad. Sci. USA 51, 786–793.
[2] Matthews, H.R. and Bradbury, E.M. (1982) in: Genetic Expression in The Cell Cycle (Padilla, G.M. and McCarty, K.S. sr eds) pp.31–54, Academic Press, New York.
[3] Doenecke, D. and Gallwitz, D. (1982) Mol. Cell. Biochem. 44, 113–128.
[4] Reeves, R. (1984) Biochim. Biophys. Acta 782, 343–393.
[5] McCarty, K.S. sr, Kelner, D.N., Wilke, K. and McCarty, K.S. jr (1982) in: Genetic Expression in The Cell Cycle (Padilla, G.M. and McCarty, K.S. sr eds) pp.55–102, Academic Press, New York.
[6] López-Rodas, G., Pérez-Ortín, J.E., Tordera, V., Salvador, M.L. and Franco, L. (1985) Arch. Biochem. Biophys. 239, 184–190.
[7] Garcea, R.L. and Alberts, B.M. (1980) J. Biol. Chem. 255, 11454–11463.
[8] DeLange, R.J., Fambrough, D.M., Smith, E.L. and Bonner, J. (1969) J. Biol. Chem. 244, 5669–5679.

[9] Arfmann, H.A. and Haase, E. (1981) Plant Sci. Lett. 21, 317–324.
[10] Nadeau, P., Pallotta, D. and Lafontaine, J.G. (1974) Arch. Biochem. Biophys. 161, 171–177.
[11] Weintraub, H., Palter, K. and Van Lente, F. (1975) Cell 6, 83–88.
[12] Spiker, S., Murray, M.G. and Thompson, W.F. (1983) Proc. Natl. Acad. Sci. USA 80, 815–819.
[13] Libby, P.R. (1978) J. Biol. Chem. 253, 233–237.
[14] Böhm, T., Schlaeger, E. and Knippers, R. (1980) Eur. J. Biochem. 112, 353–362.
[15] Libby, P.R. (1980) Arch. Biochem. Biophys. 203, 384–389.
[16] Sures, I. and Gallwitz, D. (1980) Biochemistry 19, 943–951.
[17] Wiegand, R.C. and Brutlag, D.L. (1981) J. Biol. Chem. 256, 4578–4583.