Vol. 92, No. 1, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 15, 1980 Pages 163-168

SUBSTRATE REGULATION OF HISTONE ACETYLTRANSFERASE FROM *Artemia salina*

Amparo Cano[1], Inmaculada Estepa and Angel Pestaña[2]

Instituto de Enzimologia y Patologia Molecular del C.S.I.C.
Departamento de Bioquímica
Facultad de Medicina de la Universidad Autonoma
Madrid-34, Spain

Received November 26,1979

SUMMARY. Histone H1 is the best substrate for the histone acetyltransferase from *Artemia*, while the acetylation of unfractionated histones by the isolated enzyme or the acetylation of endogenous histones in nuclear suspensions is exclusively confined to the arginine rich histones, H3 and H4. These discrepancies are explained on the basis of specific inhibition by H4 on the acetylation of H1.

INTRODUCTION

Histone acetylation within nucleosomes is increasingly viewed as one of the mechanisms for the modulation of chromatin structure and function (1-3), but little is known about the regulation of histone acetylation itself. It is well stablished (4) that the postsynthetic acetylation of histones in chromatin is restricted to a few lysines in the arginine rich histones, H3 and H4. This limited specificity of the histone acetyltransferase is particularly intriguing, in view of the fact that the other histones, specially H1, are richer in lysine residues.

We now bring electrophoretic and kinetic evidence, showing that H4 specifically inhibits the modification of H1 by the histone acetyltransferase from *Artemia*. This observation suggests a role for H4 in the modulation of histone acetyltransferase activity.

METHODS

Dormant gastrulae from Artemia (San Francisco Bay brand, Metaframe Co., Menlo Park, Cal 94025, U.S.A.) were rehydrated and grown as previously described (5). Nauplius larvae were harvested 40 hours after

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[1] A.C. present address: Department of Biology, Massachussetts Institute of Technology, Cambridge, Mass. 02139, U.S.A.

[2] To whom correspondence should be addressed.

0006-291X/80/010163-06$01.00/0

the onset of development, washed in deionized water and kept frozen at - 20° C. Homogenization, preparation of the nuclear fraction and the assay for ^{14}C acetate incorporation by nuclei have been described in detail(5).

Artemia histones were acetylated in a nuclear suspension containing 0.4 M sucrose, 5 mM mercaptoethanol, 50 mM Tris.HCl pH 7.5 and 100 nCi per ml of (1-^{14}C) acetyl coenzyme A (58 mCi/ mmol, from Amersham). After 30 min at 37° C, the reaction was stopped with 0.4 M H_2SO_4, final concentration. After stirring for 1 hour in the cold and centrifugation at 12.000 x g for 10 min, the histone sulphates were dialyzed overnight against 95 % ethanol and the resulting precipitate was collected and washed three times with cold ethanol.

Histone acetyltransferase (E.C. 2.3.1.48) was purified up to the DEAE cellulose or hydroxylapatite steps according to procedures already described (6). Acetate transfer to histones was assayed in a medium containing 20 mM Tris.HCl pH 7.5, 5 mM mercaptoethanol, 50 nCi of ^{14}C acetyl coenzyme A, acetyltransferase (about 0.05 mg of protein) and the indicated amount of histones per milliliter.The reaction was stopped with trichloroacetic, at the final concentration of 20 %, and the precipitates collected on Whatman GF/C filters for radioactivity meassurement(5) or sedimented and washed three times with cold acetone-0.05 M HCl for electrophoresis.

Whole calf thymus histones (type II-A from Sigma) were fractionated according to Oliver et al.(7) and were shown near 90% homogeneous by the electrophoretic criterium.Electrophoresis of histones was carried out by the Panyim and Chalkley method(8). The stained gels were scanned with a Gilford 2400 spectrophotometer at 600 nm.Radioactivity in slices of the gels was meassured as described(6). Protein concentration was estimated by the Lowry et al. method(9).

RESULTS AND DISCUSSION

As reported before (6), histone acetyltransferase from Artemia exhibits , in partially purified preparations, a marked preference for H1 as substrate and a relativelly small activity with arginine rich histones, especially the H4 class (the relative activities with the histones were as follows: 150 for H1, 100 for whole histone , 80 for H3 and 40 for H4). This behaviour of the isolated enzyme is in contrast to the acetylation of endogenous histones in nuclear preparations of Artemia, whose electrophoretic pattern (shown in fig 1) demonstrates a preferential modification of arginine rich histones. In order to explore these discrepancies in substrate specificity, we undertook an electrophoretic study of the reaction products of the acetyltransferase, assayed with several combinations of histones.

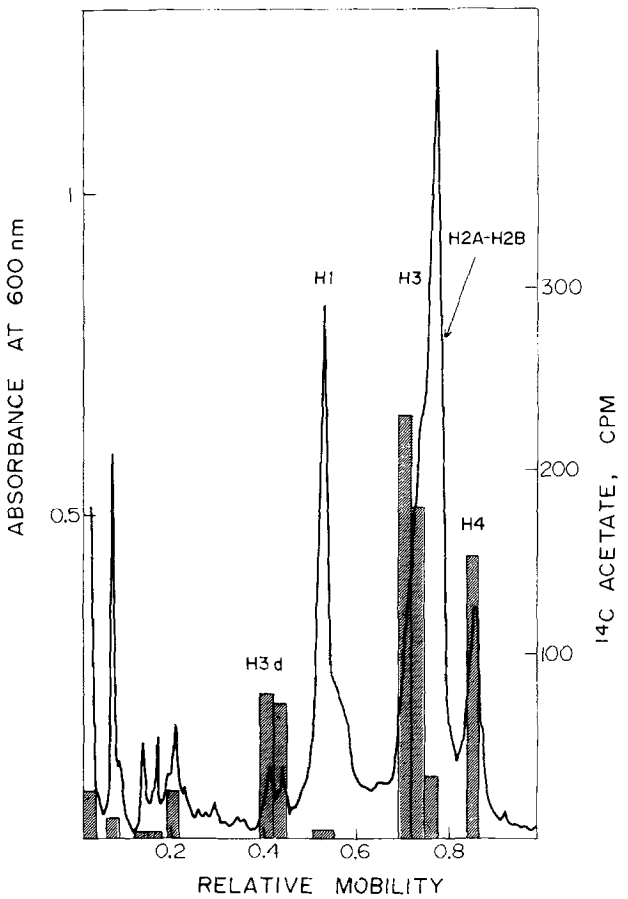

Fig 1. Electrophoretic analysis of acetylated histones from Artemia. Histones were acetylated in 2 ml of a nuclear suspension of Artemia, equivalent to 2 gm (wet weight)of animals, as described in methods. The extracted histones (0.2 mg of protein) were electrophoresed, scanned and assayed for radioactivity as described im methods.The figure shows the protein profile (adsorbance at 600 nm) and the radioactivity found in the main protein bands (shaded bars).

As shown in fig 2 A, H1 accounts for most of the acetate incorporated when this histone was given as the only substrate to the enzyme. The small radioactivity found in other sections of the gel electropherogram, represents a small contamination by other histone fractions. When the five fractions present in unfractionated calf thymus histones were given as substrate, the radioactive acetate was exclusively found in the arginine rich fractions (fig 2 B), with only traces of radioactivity in H1. An essentially similar distribution of radioactive acetate was observed when arginine rich histones were present as the only substrate acceptors

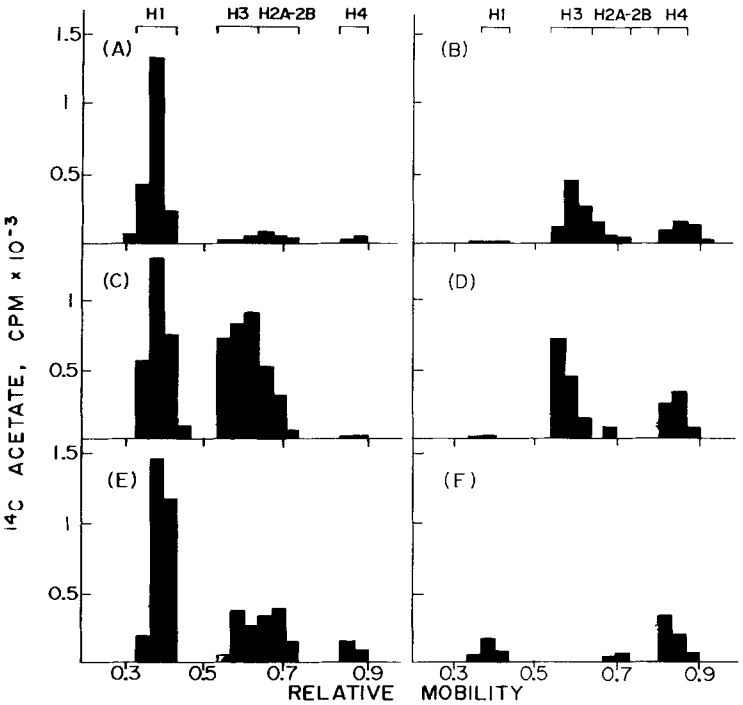

Fig 2. Electrophoretic analysis of the reaction products of histone acetyltransferase with different histone substrates. Acetate transfer to histones was assayed in 2 ml of the reaction mixture described in methods. After 20 min incubation at 35°C, the histones were precipitated with thrichloroacetic acid for electrophoretic analysis. The bars represent the net radioactivity found in the main protein bands. Histone substrates were as follows: A (H1: 0.1 mg), B (unfractionated histones:0.1 mg), C (0.05 mg of each H1 and H3), D (0.05 mg of each H3 and H4), E (H1: 0.05 mg, H2a: 0.025 mg and H2b:0.025 mg), F (0.05 mg of each H1 and H4).

(fig 2 C). These results, which confirm and extend previous observations (6), suggests that the substrate specificity found in nuclear suspensions of Artemia (fig 1), and in studies with the isolated enzyme and whole histone as substrate (fig 2 B), is the result of histone acetyltransferase regulation by its own substrates.

We next explored the influence of single histones or definite histone mixtures on the acetylation of H1. In fig 2 C it is shown that, in the presence of an equal amount of H1 and H3 (on weight basis), the acetylation of both histones is additive (5700 net cpm as compared to 2900 net cpm found with H1 alone). It is also noticeable that the net acetate incorporation in H3 is higher than that observed in the electropherogram of the H3-H4 mixture (compare fig 2 C and 2 D). This result suggests

that H4 partially inhibits the acetylation of H3. In fig 2 E it is shown that the presence of histones H2a-H2b does not affect the acetylation of H1; the small radioactivity in the H3-H4 region observed in this electropherogram represents the acetylation of arginine rich histones, contaminating the H2A-H2b preparation.

In contrast to the results above reported, the acetylation of H1 was almost completely supressed when assayed in the presence of an equal amount (on weight basis) of H4 (fig 2 F), while this histone was modified to a similar extent to that found in H3-H4 mixtures (fig 2 D) or unfractionated histones (fig 2 B).

In order to confirm the pressumptive role of H4 as an inhibitor of the acetylation of H1, as suggested by the electrophoretic study, we next assayed the histone acetyltransferase activity with H1 as substrate, in the presence of several concentrations of H4. The assay conditions were chosen so as to obtain initial velocities (6) and minimal acetylation of

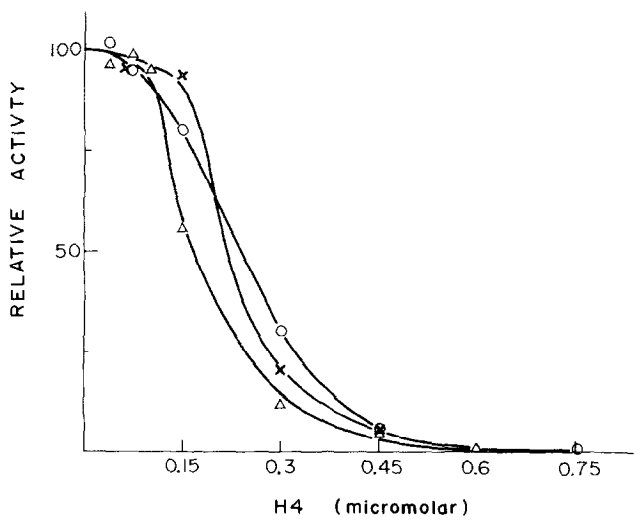

Fig 3. Inhibition of histone acetyltransferase by H4. Histone acetyltransferase (hydroxylapatite step) was assayed with H1 as substrate in the presence of the concentrations of H4 indicated in the figure. Incubations in a final volume of 0.5 ml were for 10 min at 35°C. The radioactivity insoluble in thrichloroacetic acid was meassured as described in methods, after the addition of 0.05 mg of carrier histones. Relative activities were obtained after correction for the small contribution of H4 to the total radioactove incorporation. The concentration of H1 for each set of inhibition curves was: 5×10^{-7}M (●), 10^{-6}M (▲) and 2.5×10^{-6} M (✕)

H4 (less than 200 cpm for the maximal concentration of H4, against 2500 cpm for the incorporation in H1 at the lower concentration). It can be seen in fig 3 that increasing concentrations of H4 results in a close family of S-shaped inhibition curves, at any of the concentrations of H1 assayed. These results suggest that H4 acts as an allosteric , non-competitive inhibitor of the acetylation of H1, with an $I_{0.5}$ close to 2×10^{-7} M.

We conclude from these experiments that the substrate specificity of the histone acetyltransferase from Artemia is regulated by the histone H4, which in turn is one of its substrates. Such a regulation by substrate allows us to reconcile the high activity with H1 of the acetyltransferase from Artemia (6) with the known specificity of histone acetylation in vivo (4) and in vitro, with intact nuclei (fig 1). This inhibition may serve as a regulatory mechanism by which the acetate transfer to histones can be directed to a specific acceptor molecule.

ACKNOWLEDGEMENTS : We thank C.Gancedo,J.Mª Sempere, J.Renart and C.F. Heredia for critical reading of the manuscript. This work was financed in part by a Grant from the J.March Foundation. A.C. and I.E. were P.F.P.I fellows from the Spanish Ministry of Education and Science.

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