CHARACTERIZATION OF A CYTOPLASMIC HISTONE–COENZYME A ACETYLTRANSFERASE[1]

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Received 28 June 1971
Revised version received 23 August 1971

1. Introduction

Enzyme activities catalyzing the transfer of the acetyl radical from acetyl CoA to proteins have been described in rat liver and brain nuclei [1, 2]. These enzymes, apparently specific for histones, can account for the acetylation of internal N-lysine residues [3] within preformed histones [4], a process which seems to take place within the nucleus [5]. On the other hand, the presence of N-terminal acetyl residues in a variety of non-histone proteins, and the occurrence of an N-terminal acetylation apparently related to polypeptide chain initiation [6, 7] requires the occurrence of acetyltransferase activity in other parts of the cell. Studies described herein demonstrate the presence in liver cytoplasm of a histone–coenzyme A acetyltransferase activity which is significantly different in a number of characteristics from the nuclear enzyme.

2. Methods and materials

Female rats obtained from the Holtzman rat farm in Madison, Wisc., USA, were utilized for these experiments. The animals were decapitated and the liver quickly removed and cooled in an ice bath. Homogenates of the liver were prepared with a glass-teflon homogenizer in 0.35 M sucrose in 0.05 M Tris-HCl pH 7.4 containing 0.025 M KCl and 0.04 M $MgCl_2$. After centrifuging at 1000 *g* for 10 min, the supernatant was collected and spun at 50,000 rpm for 1 hr in the 50.1 (Spinco) rotor to prepare a soluble cytoplasmic fraction. The nuclei were purified from the 1000 *g* pellet essentially as described by Gallwith [1]. The resulting pellet was washed 2 times and resuspended in 0.05 M Tris-HCl pH 7.4. The precipitate which forms after standing at 0° for ½–1 hr was removed by low speed centrifugation and the resulting clear supernatant which contained about 1 mg protein per ml used as a source of the nuclear enzyme.

Histone acetylation was assayed in duplicate as described by Bondy et al. [2] with minor modifications. The standard assay mixture contained in 0.5 ml, 100 μg of histone type II, 80–100 μmoles of KCl, 50 μmoles of Tris-HCl pH 7.4, 10–20 μg of liver protein and 0.020 μCi 1-^{14}C-acetyl CoA. The reaction was started by the addition of radioactive acetyl CoA and was stopped after 10 min at 37° by the addition of 3–4 ml of 20% cold TCA. Controls without histone or with heated (for 10 min at 80°) enzyme were run in parallel. The TCA insoluble material was collected on Millipore membranes and washed free of soluble ^{14}C-acetate as described [2]. The radioactivity retained on the membranes was measured in a Packard Tri Carb scintillation spectrometer.

[1] This work was supported in part by grants from the National Cancer Institute (CA-07175) and the American Cancer Society (E-588).
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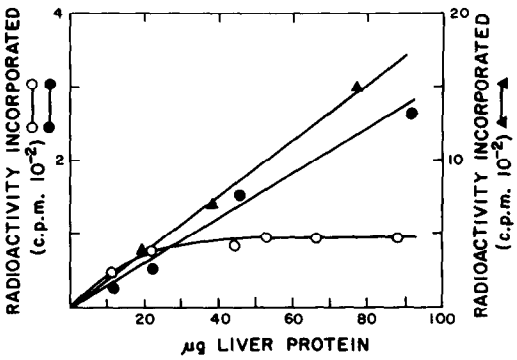

Fig. 1. The influence of increasing liver protein concentration on the incorporation of ^{14}C-acetate into histones. Three different enzyme sources were used: crude liver cytoplasm (○—○), dialyzed liver cytoplasm (●—●) and the dialyzed 20–40% ammonium sulfate precipitate (▲—▲). Dialysis was carried out overnight at 0° against 0.05 M Tris-HCl pH 7.5, containing 0.001 M dithiothreitol.

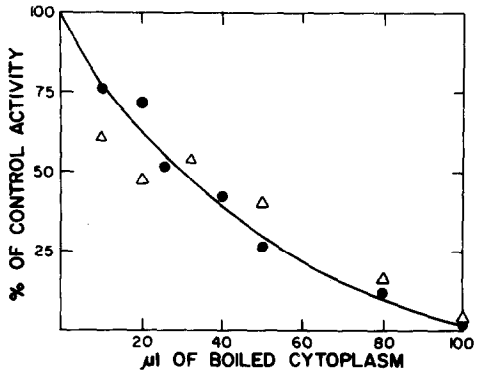

Fig. 2. Inhibition of acetylating activities in nuclei and cytoplasm by boiled liver extract. The boiled extract was prepared from the post nuclear (1000 *g*) supernatant, kept in boiling water for 10 min and then freed of coagulated proteins by centrifugation. Histone acetylating activity of nuclear (△—△) and cytoplasmic (●—●) preparations was assayed in the presence of variable amounts of the boiled extract; and the resulting activities plotted as a per cent of the control values (no boiled extract addition). In this experiment the fraction which precipitates from 20–40% ammonium sulfate was used as a source of the cytoplasmic enzyme activity.

The difference in ^{14}C-acetate incorporation obtained with the native enzyme preparation and the heat denatured enzyme preparation was taken as due to enzymatic acetylation. This allows one to correct for the spontaneous histone acetylation [8], which is very low at pH 7.5 in the presence of 0.1 M potassium [9]. The histone acetylating activity was obtained by subtracting from the radioactivity of the samples with histone the counts incorporated into histone-free controls.

^{14}C-acetyl CoA was obtained from the New England Nuclear Corp. and had a specific activity of 58.3 mCi/mmole. Calf thymus histones type II, III (lysine-rich) and IV (arginine-rich) were obtained from the Sigma Chemical Company, St. Louis, Mo. Other chemicals utilized in these experiments were of the highest grade obtainable.

3. Results

Rat liver cytoplasm catalyzes the transfer of ^{14}C-acetate from acetyl CoA to histones; however, in crude preparations, the acetylating activity does not show linearity with time or increasing protein concentration. Upon dialysis of the cytoplasmic fraction, linearity of enzyme activity could be obtained up to

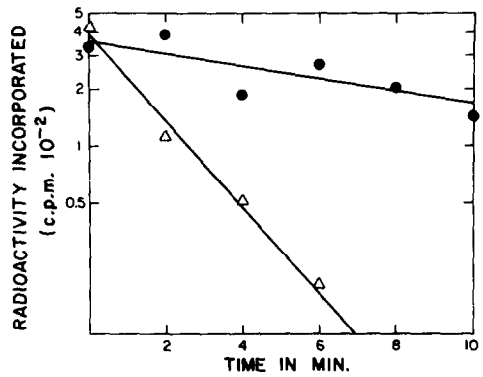

Fig. 3. Thermal inactivation of histone acetylating activities. The ammonium sulfate precipitates (20–40%) were used as the enzyme source from both nuclei and cytoplasm. The preparations were diluted in 0.05 M Tris-HCL pH 7.5 to give suspensions of similar protein concentration (1.1 mg/ml) and roughly the same specific activities. The diluted preparations were incubated at 47.5°, and at the indicated time intervals, samples were taken and quickly cooled for the assay of acetylating activity. Symbols are the same as those of fig. 2.

Table 1
Characteristics of nuclear and cytoplasmic histone acetyltransferase from rat liver.

	Nucleus	Cytosol
Units/g liver[1]	5 × 10^3	25 × 10^3
Specific activity in the crude extract	4.2 × 10^3	0.5 × 10^3
Specific activity in 40% $(NH_4)_2SO_4$ precipitate	16.4 × 10^3	13 × 10^3
Specific activity in pH 5 precipitate[2]	14 × 10^3	9 × 10^3
Ratio H/E activities[3]	10	1
Ratio of lysine-rich/arginine-rich histone acetylation	0.27	1.01
Activity after 4 min[4] at 47.5° as percent of control	10	75

[1] Counts/min incorporated into histone under the assay conditions (10 min at 37°). The units per gram and the specific activity (units/mg protein) in the crude cytoplasmic extract were measured after dialysis as indicated in the legend to fig. 1.

[2] The crude nuclear and cytoplasmic preparations were made to pH 5.1 by the dropwise addition of 0.2 M acetic acid with vigorous stirring. After 30 min, the precipitate was collected by centrifugation (30 min at 10,000 rpm) and resuspended in a small amount of 0.05 M Tris-HCl pH 7.5 and its activity determined.

[3] H indicates the incorporation of acetyl into histone while E is the radioactivity incorporated into the unidentified TCA insoluble material which can be detected in the assay carried out in the absence of histone.

[4] Calculated from the experiment described in fig. 3.

100 μg of protein (fig. 1); the acetate incorporation being also linear with time up to 30 min under these conditions. These results suggested the presence of a dialyzable inhibitor in the cytoplasmic fraction.

In fig. 2 is demonstrated the fact that by addition of an extract of boiled homogenate, inhibition of both the nuclear and cytoplasmic activities of the acetyltransferase occurred. Upon the addition of 0.1 ml of boiled extract, virtually 100% inhibition of the enzymatic activity was obtained. These findings might then account for the failure of previous workers [1, 2] to demonstrate the presence of a cytoplasmic histone acetyltransferase, especially since preliminary investigations in our laboratory suggested that the nuclei themselves probably do not contain this inhibitor.

Thus, in view of the fact that cytoplasmic histone acetyltransferase activity could be demonstrated, we then proceeded to determine some of its characteristics. In table 1 is seen a tabulation of the various characteristics of the two enzymes. The total units per gram of liver suggest that the enzyme in the cytosol is more active than that in the nucleus by a factor of 5. On the other hand, because of the much greater amount of protein in the cytosol, its specific activity in a crude extract is considerably less. Fractionation of both the cytoplasmic and nuclear preparation results in essentially similar separations of the acetylase activity by ammonium sulfate and isoelectric precipitation. The activity toward the lysine-rich and arginine-rich histones was considerably different for the two enzymes. Whereas the cytosol enzyme had roughly the same activity on the two histones, the nuclear enzyme definitely preferred the arginine-rich fraction. Perhaps the most striking difference between the two enzymes was their heat stability as demosntrated in fig. 3. Within 6 min of heating at 47°, the nuclear enzyme had been completely inactivated, whereas only 20–30% of the cytoplasmic enzyme activity was lost during this time period.

4. Discussion

The studies presented herein demonstrate the existence of a cytoplasmic histone–coenzyme A acetyltransferase activity which differs in several respects from the activity found in nuclei. While the exact function of a cytoplasmic histone acetyltransferase is not clear at this time, it is certainly apparent that many cytoplasmic enzyme proteins do have acetyl groups as part of their overall structure. Previous suggestions from this laboratory [10] have indicated that acetyltransferase enzymes may be akin to protein kinases with respect to the possibility of cyclic nucleotide activation. Studies on the cytoplasmic histone acetyltransferase are presently under way in an attempt to determine whether or not this enzyme has a specific function in the acetylation of certain cytoplasmic enzymes, notably serine dehydratase and whether or not such function may be modified by the presence of cyclic nucleotides.

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