ACETYLATION OF HISTONES BY A KINASE FROM RAT LIVER NUCLEI

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A variety of proteins have been reported to contain N-acetyl groups (Harris, 1959, Harris and Hindley, 1961, Margoliash et al., 1961, Marshall and Neuberger, 1961, Narita, 1958, Phillips, 1963, Satake et al., 1963, Schroeder et al., 1962, Titani et al., 1962). The reactions which form terminal acetyl groups in proteins and their biological significance are not understood. The detction of acetyl groups in calf thymus histones (Phillips, 1963) and the finding that chemically acetylated histones are less effective inhibitors of the DNA-dependent RNA polymerase in vitro led to the hypothesis that acetylation of histones could be important in the regulation of RNA synthesis (Allfrey et al., 1964, Pogo et al., 1966). Isolated calf thymus (Allfrey et al., 1964) and rat liver nuclei (Gallwitz and Sekeris, in prep.) acetylate histones in vitro and enzymes which incorporate C^{14}-acetate into histones have been demonstrated in pigeon liver (Nohara et al., 1966).

This paper reports the transfer of acetate from coenzyme A to rat liver histones by an acetokinase from pure rat liver nuclei.

Methods and Materials

Liver nuclei were isolated from male 150-200 g BR II rats by a modification of the procedure of Chauveau et al. (1956). A 30 % (v/v) homogenate was made in ice cold buffer consisting of tris-HCl 0.05 M, pH 7.5, sucrose 0.25 M,

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KCl 0.025 M and $MgCl_2$ 0.01 M (TSS) with use of a motor driven Potter-Elvehjem homogenizer. The 1000 x g pellet was suspended in 2.2 M sucrose made in TSS and centrifuged for 1 hour at 48,000 x g. The nuclear pellet was then washed once more in ice cold TSS.

Histones of rat liver nuclei were isolated by method II of Johns (1964), the acetylating enzyme from an acetone powder according to Chou and Lipmann (1952). Two fraction were obtained, one at an acetone concentration of 40 % (fraction A) and one at 60 % (fraction B).

For the estimation of acetokinase activity, histones were incubated with varying amounts of enzyme proteins at 37 C in a final volume of 0.5 ml in capped glass tubes in 0.08 M tris-HCl buffer at different pH values in the presence of 0.025 µC acetyl-1-C^{14}coenzyme A. The reaction was stopped by precipitating the incubation mixtures onto filter paper discs (Schleicher and Schüll, 2043 b paper) in ice cold 15 % TCA. After standing 20 min the paper discs were heated at 90 C in 15 % TCA for 15 min, washed once more in TCA, treated with absolute ethanol, ethanol-ether and ether and the radioactivity of the dried discs counted in a Nuclear Chicago liquid scintillation counter.

Acetyl-1-C^{14}coenzyme A (spec. act. 54.5 mC/mM) was obtained from New England Nuclear Corp., bovine serum albumin and human serum γ-globulin from Serva, Entwicklungslabor, Heidelberg. Ribonuclease was a product from Worthington Biochem. Corp.

Protein was estimated according to Lowry et al. (1951).

Results

The data in Table I show that both enzyme fractions A and B from pure rat liver nuclei catalyze the transfer of acetate from acetyl-1-C^{14}coenzyme A to rat liver histones; fraction B is about twofold more active. The acetate transfer is Mg^{++}-independent and moreover, 1 mM Mg^{++} markedly inhibitory.

Histone fraction f3 was found to be a better in vitro acetate acceptor than fractions f2a and f1. Acetate was not incorporated into the enzyme itself.

Table I. Acetylation of histones by nuclear enzymes. Reactions run for 18 min at pH 7.7 with 0.5 mg histone and 9 µg enzyme protein as described under methods.

Histone	Enzyme	Acetate Incorporation
		µµmoles/mg enzyme protein
f3	fr B	11.34
"	" (Mg^{++} 1 mM)	6.19
"	" (65 C, 5 min)	-
-	"	-
f2a	"	8.75
f1	"	4.12
f3	fr A	6.39

Table II. Effects of pH on incorporation of acetate into histone fraction f3 and on acetokinase activity *in vitro*. Incubations run for 15 min with 0.5 mg histone and 5 µg enzyme protein as described under methods.

Histone	Enzyme	pH 7.7	pH 9.0	
f3	-	0.06	1.12	Acetate Incorp. (µµmoles)
"	fr B	4.54	-	" " (" /mg enzyme protein)

After storage of enzyme fractions A or B at -35 C for 24 hours in 0.1 M tris-HCl buffer, pH 7.5, slow thawing resulted in markable losses of enzyme activity. The acetokinase activity was completely destroyed at 65 C.

Rat liver histones incubated *in vitro* with labelled acetyl coenzyme A incorporate acetate into a heat stable form. The process increases sharply on rising the pH. In Table II the acetylation of the histone fraction f3 is compared at pH 7.7 and pH 9.0 in the presence and absence of the acetokinase from rat liver nuclei. The enzyme is inactive at pH 9.0.

To test the specificity of the nuclear acetokinase, the transfer of acetate to different proteins was measured, Table III. Although all histone fractions tested were acetylated in the presence of the enzyme, very little acetate was incorporated into pancreatic ribonuclease and none into bovine serum albumin and human serum γ-globulin.

Table III. Acetylation of different proteins by nuclear kinase. Incubations run for 15 min at pH 7.7 with 0.5 mg of the different proteins and 5 µg enzyme protein as described under methods.

Protein	Enzyme	Acetate incorporation
		µµmoles/mg enzyme protein
Histone fraction f3	fr B	4.54
Pancreatic RNase	"	1.51
Bovine serum albumin	"	-
Human serum γ-globulin	"	-

Discussion

Enzymes which catalyze the transfer of acetate to proteins have been demonstrated in reticulocytes and in pigeon liver (Marchis-Mouren and Lipmann, 1965, Nohara et al., 1966). Acetate transfer from acetyl coenzyme A to haemoglobin by the reticulocyte enzyme was found to be rather nonspecific. No acetate transfer to calf thymus histones was seen. Acetone fractions of whole pigeon liver prepared as in the present paper were reported to contain both acetate activating and transferring enzymes. The enzymes required Mg^{++} ions. In our study with acetyl coenzyme A as precursor the acetylation of histones by the acetokinase from rat liver nuclei was not Mg^{++} ion dependent, thus suggesting that the Mg^{++} requirement in the pigeon liver system may be only for acetate activation.

The acetylation of histones in isolated rat liver nuclei was found to increase on rising the pH from 7.5 to 9.0 (Gallwitz and Sekeris, in prep.). The present investigation indicates clearly that at pH 9.0 the incorporation of acetate into histones is a chemical rather than an enzymatic reaction. The enzyme fraction prepared from liver nuclei catalyzes the acetylation not only of histones but also under the same conditions to a smaller extend to pancreatic ribonuclease, a protein which does not contain N-acetyl groups. Bovine serum albumin and human serum γ-globulin are not acetylated. Further work must be done to determine wether there are specific protein acetokinases.

Although the function of acetyl groups in histones is subject to speculation, there are some indications that this acetylation could be important in the process of RNA synthesis (Allfrey et al., 1964, Gallwitz and Sekeris, in prep., Pogo et al., 1966). It is therefore interesting that enzymes needed for such histone modifications are found in the cell nucleus.

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