ENZYMATIC DEACETYLATION OF HISTONE

Akira Inoue and Daisaburo Fujimoto

Department of Chemistry, Faculty of Science,
Tohoku University, Sendai, Japan

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SUMMARY

An enzyme activity which catalyzed the deacetylation of histone was found in the calf thymus extract. ^{14}C-Acetyl-histone prepared by incubating calf thymus nuclei with sodium^{14}C-acetate served as the substate and treatment of the histone with pronase destroyed most of the susceptibility to the enzymatic deacetylation. ^{14}C-Acetyl-histone prepared by chemical acetylation with ^{14}C-acetic anhydride served as the substrate to a less extent.

Acetyl groups are present in many histone fractions and they are attached either to the amino-terminal nitrogen of the polypeptide chain(Phillips, 1963 ; 1968) or to the ε-amino groups of lysine residues(Gershey et al., 1968 ; Vidali et al.,1968 ; DeLange et al.,1969). Turnover of these acetyl groups in histones is observed in the mammalian cells and the rate varies among various tissues(Pogo et al.,1968 ; Byvoet ,1968). It has been proposed that the structural modifications of histones by acetylation and deacetylation would influence the interactions between histones and DNA and might play a role in regulation of information transfer from DNA (Allfrey et al.,1964).

This paper reports that the calf thymus extract contains an enzyme activity which catalyzes deacetylation of histone.

METHODS

Calf thymus (2.1 g) was minced and homogenized in 0.14M NaCl (5.0 ml)

in a Potter-Elvehjem type homogenizer for 3 min. The homogenate was centrifuged at 10000xg for 15 min and the supernatant was used as the enzyme preparation.

Two kinds of ^{14}C-acetyl histones were prepared. "Biologically acetylated" histone was prepared by incubating calf thymus nuclei with sodium 1-^{14}C-acetate(20 μC / ca. 0.3 g nuclei, specific activity 40 mC / m mole) according to the method of Gershey et al.(1968). Whole histone was extracted from the nuclei with 0.25M HCl(MacGillivray ,1968). "Chemically acetylated" histone was prepared by acetylation of histone with ^{14}C-acetic anhydride. Whole histone sulfate(Murray ,1964) from calf thymus(60 mg) was treated with 1-^{14}C-acetic anhydride(20 μC ,1.1 μ mole) in 2.5 ml of 2M sodium acetate pH 8.1 at 0° for 4 hours. After the treatment, histone was recovered by precipitation with ethanol(5 ml) and washed throughly with ethanol.

For the assay of the enzyme activity, ^{14}C-acetyl histone(2.5 mg) was incubated with the enzyme preparation(4.0 mg protein) at 37° in a final volume of 0.6 ml in 0.025M Tris-HCl buffer, pH 7.3. After the incubation, the reaction mixture was acidified by the addition of 0.1 ml 0f 0.1M HCl containing 5μ mole of carrier acetic acid. Acetic acid was extracted by shaking with 3 ml of ethylacetate. Two ml of the organic layer was taken, mixed with the scintillator solution and the radioactivity was measured by a Kobe Kogyo liquid scintillation counter, Model GSL-111. Values were corrected for the efficiency of the extaction with ethylacetate(80 %) and for the efficiency of the counting(ca. 80 %).

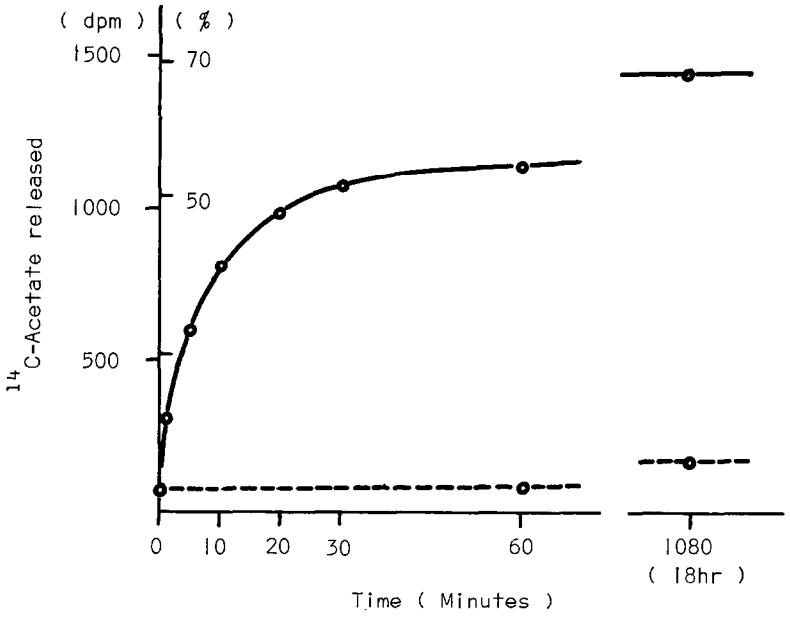
RESULTS and DISCUSSION

Fig. 1 shows the time course of the deacetylation reaction when "biologically acetylated" histone was incubated with the calf thymus extract. After 60 min incubation, about 55 % of the ^{14}C-acetyl groups was released. Without the enzyme, no significant deacetylation occurred.

In order to confirm the liberation of ^{14}C-acetate and to check the possible

Fig. 1

Time course of the deacetylation



The "biologically acetylated" histone (2.5 mg, 2100 dpm) was incubated with (o——o) or without (o---o) calf thymus extract at 37° .

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contamination of ^{14}C-acetyl-amino acids or ^{14}C-acetyl-peptides in the ethyl-acetate extract, acetic acid was seperated by azeotropic distillation from the ethylacetate extract. Radioactivity in the distillate(i.e.^{14}C-acetic acid) accounted for about 90 % of the ethylacetate extractable radioactivity.

As shown in Table 1, treatment of the ^{14}C-acetyl-histone with pronase destroyed most of the susceptibility to the enzymatic deacetylation. The results indicate that the deacetylase attacks the acetyl groups in a large molecule and is different from other known aminoacylases. The results ruled out, therefore, the possibility that the acetate liberation from histone resulted from the cooperation of proteases with aminoacylase(E.C.3.5.1.14) or acyl-lysine deacylase(E.C.3.5.1.17).

Deacetylation rate of the "biologically acetylated" histone and that of

Table 1

Comparison of the deacetylation of the histone preparations

Substrate	Activity in substrate (dpm)	Incubation time (min)	^{14}C-Acetate released Activity (dpm)	Degree of deacetylation (%)
"Biologically acetylated"histone	2100	0	97	5
		10	912	44
		30	1017	49
Pronase-digested "biologically acetylated"histone*	2100	0	36	2
		10	133	6
		30	157	7
"Chemically acetylated"histone	10000	0	349	3
		10	524	5
		30	888	9

^{14}C-acetyl-histone(2.5 mg) was incubated with the calf thymus extract at 37°. * --- The histone(2.5 mg) was digested with pronase(0.014 mg) in 0.3 ml of 0.05M Tris-HCl buffer ,pH 7.3, at 37° for 60 min. After the digestion, the solution was heated at 100° for 15 min and served for the deacetylation reaction.

the "chemically acetylated" histone were compared(Table 1).

After 10 min incubation, 44 % of ^{14}C-acetyl groups in the "biologically acetyl ated" histone was released while only 5 % of ^{14}C-acetyl groups in the case of the "chemically acetylated" histone. In the "biologically acetylated" histone, ^{14}C- acetyl groups are known to attach to ε-amino groups of the specific lysine residues(Vidali et al.,1968 ; DeLange et al.,1969). The "chemically acetylated" histone has not been characterized, but ^{14}C-acetyl groups are probably introduced non-specifically into α-amino groups of N-terminal amino acids and ε-amino groups of a number of lysine residues. The deacetylase may have a high degree of specificity and distinguish some specific acetyl-amino groups in the histone molecule

Although the function of acetyl groups in histone is not yet clear , there are some indications that acetylation and deacetylation are involved in

the activation and repression of the gene (Allfrey et al., 1964; Pogo et al., 1968). The deacetylase demonstrated here as well as histone acetylases (Nohara et al.,1968; Gallwitz,1968) may play an important role in genetic control mechanism in higher organisms.

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REFERENCES

Allfrey, V.G., Faulkner, R., and Mirsky, A.E., Proc. Nat. Acad. Sci. U.S., 51, 786 (1964)
Byvoet, P., Biochim. Biophys. Acta, 160, 217 (1968)
DeLange, R.J., Fambrough, D.M., Smith, E.L., and Bonner, J., J. Biol. Chem., 244, 319 (1969)
Gallwitz, D., Biochem. Biophys. Res. Commun., 32, 117 (1968)
Gershey, E.L., Vidali, G., and Allfrey, V.G., J.Biol. Chem., 243, 5018 (1968)
MacGillivray, A.J., Biochem. J., 110, 181 (1968)
Murray, K., Biochemistry, 3, 10 (1964)
Nohara, H., Takahashi, T., and Ogata, K., Biochim. Biophys. Acta, 154, 529 (1968)
Phillips, D.M.P., Biochem. J., 87, 258 (1963) ; *ibid.*,107, 135 (1968)
Pogo, B.G.T., Pogo, A.O., Allfrey, V.G., and Mirsky, A.E., Proc. Nat. Acad. Sci. U.S., 59, 1337 (1968)
Vidali, G., Gershey, E.L., and Allfrey, V.G., J. Biol. Chem., 243, 6361 (1968)