ENZYMATIC DEACETYLATION OF f2a2 HISTONE

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1. Introduction

Acetylation of ε-amino groups of lysine residues is observed in f2a1 and f3 histones from calf thymus [1]. In these histone fractions, the acetylated lysine residues lie in the basic amino-terminal regions of the molecules [2, 3]. It has been suggested that these regions may be DNA-binding sites and that acetylation of the ε-amino groups of the lysine residues would affect the tightness of the binding of these regions to DNA and thus participate in the regulation of information transfer from DNA [4]. Acetylation is reversible and histone deacetylases which catalyze the hydrolytic cleavage of these acetyl groups were extracted and purified from calf thymus [5–9].

Recently amino acid sequence of f2a2 histone from calf thymus was determined [10]. This histone has also a basic amino-terminal region and its sequence is homologous to the corresponding region of f2a1, but no lysine residues are acetylated significantly [10]. It may be possible that f2a2 histone is acetylated *in vivo* but the acetyl groups turn over rapidly by the action of deacetylase. However, the enzymatic deacetylation of f2a2 histone has not been examined for lack of the acetylated form.

We report here that the chemical acetylation of f2a2 with acetyl-CoA introduced acetyl groups preferentially into the ε-amino group of lysine residues in the amino-terminal region and that the introduced acetyl groups could be removed by deacetylase in the calf thymus extract.

2. Methods

f2a2 Histone was prepared from calf thymus by the method of Johns [11] and purified by Biogel P-60 column chromatography [12]. The purity was checked by polyacrylamide gel electrophoresis [13] and amino acid analysis.

Acetylation of f2a2 with [^{14}C]acetyl-CoA was carried out according to the method of Paik et al. [14]. Histone (3.75 mg) was incubated with [1-^{14}C]acetyl-CoA (0.25 μCi, 46 nmoles, the product of the Radiochemical Centre, England) at pH 9.2 at 37° for 150 min [7]. The specific activity of the product was about 5×10^4 dpm/mg, indicating about 0.06 mole of acetyl group was introduced into 1 mole of histone on an average.

The calf thymus extract was prepared as described previously [8]. Biologically acetylated whole histone was prepared by incubating calf thymus nuclei with [^{14}C]acetate [6]. Release of [^{14}C]acetate from histone was measured as described previously [6].

Complete acetylation of f2a2, trypsin digestion and fractionation of the digest were carried out according the method of Candido and Dixon [12].

3. Results and discussion

Histone fractions could be acetylated with acetyl-CoA nonenzymatically. Paik et al. found that the acetylation sites are ε-amino groups of lysine residues and at the maximum approximately one out of every 18 lysine residues is acetylated [14]. However, the lysine residues acetylated under the condition have not been characterized. In order to examine the position of

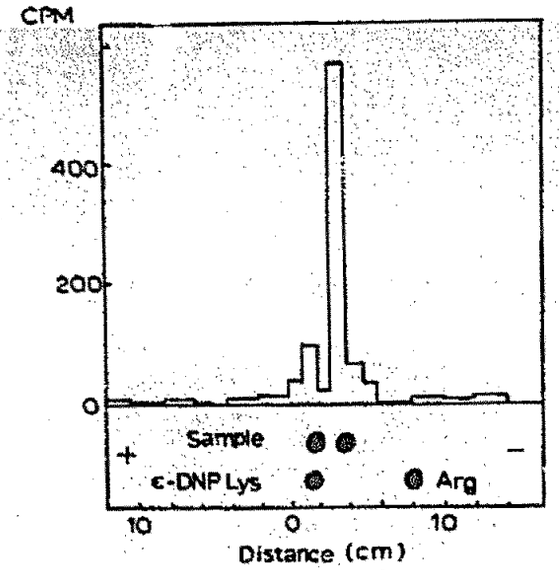

Fig. 1. Electrophoretic pattern of radioactivity of tryptic peptides. A mixture of [^{14}C]acetyl-f2a2 (0.51 mg, 38,300 dpm) and cold f2a2 (5 mg) was acetylated with acetic anhydride, digested with trypsin (0.07 mg, twice-crystallized trypsin from Worthington Biochemical Corp. was treated with diphenylcarbamoyl chloride) at 40° for 3.5 hr and the digest was fractionated on a Sephadex G-25 column (1.2×144 cm) according to the method of Candido and Dixon [12]. The radioactive peptides were applied on Toyo No. 50 filter paper (40×10 cm) and submitted to electrophoresis at 47 V per cm for 30 min in 0.1 M pyridine–acetate buffer, pH 3.6. A portion of the paper was cut into strips, 1–2 cm in length, and measured for radioactivity.

Table 1

Deacetylation of [^{14}C]acetyl-f2a2 histone by calf thymus extract.

Amount of enzyme (mg protein)	[^{14}C]Acetate released (dpm)	Degree of deacetylation (%)
0	0	0
0.12	466	16
0.24	1191	42
0.48	1768	62
0.48*	475	17
0.48**	1628	57

Incubation mixture contained calf thymus enzyme, 55 μg, 2850 dpm of [^{14}C]acetyl-f2a2, 200 μg of cold f2a2 and 10 μmoles of sodium phosphate buffer, pH 7.0. Total volume was 0.6 ml. Incubation was carried out at 37° for 60 min.

* Without cold f2a2.
** 100 μg of protamine was added in place of cold f2a2.

[^{14}C]acetyllysine residues in [^{14}C]acetyl-f2a2, the histone was first completely acetylated with cold acetic anhydride and then digested with trypsin [12]. f2a2 Histone contains 14 lysines and tryptic digestion of the completely acetylated f2a2 is expected to give 5 lysine-containing peptides, i.e. two basic peptides which involve 4 lysines located in the amino-terminal region, two neutral peptides which involve 3 lysines in the middle region and a large insoluble peptide which involves 7 lysines in the carboxyl-terminal region [10]. When the completely acetylated sample was digested with trypsin, about 95% of the radioactivity was found in the soluble fraction. This fraction was then applied on a Sephadex G-25 column (1.2×144 cm, void volume 67 ml) and eluted with 0.1 M acetic acid [12]. Most of the radioactivity (ca. 80%) was eluted between 97 ml and 106 ml. These fractions were collected, concentrated and subjected to paper electrophoresis. Two ninhydrin positive spots, one neutral and one basic, appeared and the radioactivity was located predominantly in the basic peptide fraction (fig. 1). The amino acid composition of the fraction was Lys 3.8, Arg 2.1, Thr 0.9, Glu 1.4, Gly 3.0, Ala 3.0. Therefore, it seems a mixture of

$$\overset{4}{\underset{\text{Ac}}{\text{Gly–Lys}}}\text{–Gln–Gly–Gly–}\overset{}{\underset{\text{Ac}}{\text{Lys}}}\text{–Ala–}\overset{11}{\text{Arg}}\text{ and}$$

$$\overset{12}{\underset{\text{Ac}}{\text{Ala–Lys}}}\text{–Ala–}\overset{}{\underset{\text{Ac}}{\text{Lys}}}\text{–Thr–}\overset{17}{\text{Arg}}$$

[10]. The result indicates that the principal [^{14}C]acetylated lysine residues are located in the amino-terminal region.

Deacetylation of [^{14}C]acetyl-f2a2 by the extract from calf thymus was examined. As shown in table 1, [^{14}C]acetyl groups in the histone could be removed enzymatically. Addition of cold f2a2 apparently stimulated the release of [^{14}C]acetate, but this effect nonspecific and other basic proteins such as protamine were also effective. Basic proteins probably removed some inhibitors which are present in the crude extract [8, 9].

In an attempt to examine the relation between the deacetylase for f2a2 and that for biologically acetylated f2a1 and f3, the crude extract was chromatographed on a Sepharose 4B column and fractions were assayed for the deacetylation of f2a2 and biologically

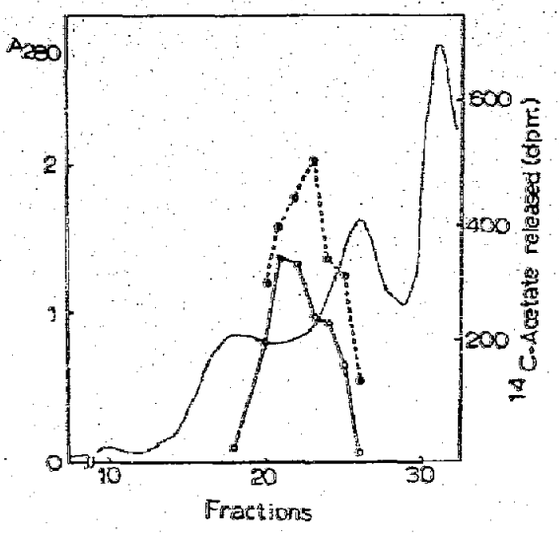

Fig. 2. Gel filtration on Sepharose 4B. The crude extract of calf thymus (148 mg protein, 10 ml) was applied on a Sepharose 4B column (2.2 × 91 cm) and eluted as described previously [8]. Fractions of 10 ml were collected. The deacetylase activity was assayed by incubating 0.5 ml aliquots with biologically acetylated whole histone (1000 dpm, 500 μg) or 0.2 ml aliquots with chemically acetylated f2a2 histone (3830 dpm, 51 μg, containing 100 μg of protamine) at 37° for 10 min. (——): Absorbance at 280 nm; (o—o—o): [^{14}C]acetate release from chemically acetylated f2a2; (•--•--•): [^{14}C]acetate release from biologically acetylated whole histone.

labeled whole histone. As shown in fig. 2, elution profiles of both activities were similar but not identical. It is not clear whether a deacetylase specific for f2a2 is present, but the result is consistent with the previous observation that histone deacetylase is heterogeneous with respect to molecular weight and substrate specificity [8].

The results described here suggest that f2a2 histone may be acetylated *in vivo* and the acetyl groups may turn over rapidly by the action of deacetylase in calf thymus.

The chemically acetylated f2a2 may be useful as a substrate for the study of histone deacetylase. However, in contrast with f2a2, the chemical acetylation of f2a1 apparently did not introduce acetyl groups into the amino-terminal region [15] and the acetylated f2a1 could not serve as a substrate for deacetylase [7].

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