SITES OF 'IN VITRO' ENZYMATIC ACETYLATION OF HISTONE H4

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1. Introduction

In the preceding paper the sites of 'in vitro' enzymatic acetylation of histone H3 have been described [1]. This report deals with the acetylation sites of histone H4. Sequential degradation of the intact histone to reveal internal ε-*N*-acetylated lysines is not possible because the N-terminal serine has a blocked, acetylated α-amino group [2]. However DeLange et al. [2] have described a peptide produced via chymotryptic and tryptic digestion which extends from residue 4–17 and contains four lysine residues, potential sites of 'in vitro' acetylation. We have isolated this peptide from 'in vitro' enzymatically acetylated histone H4 and determined the incorporation of acetate into the various lysine residues.

2. Materials and methods

α-Chymotrypsin (48 U/mg) and DCC-treated trypsin (Type II) were obtained from Worthington and Sigma respectively. Maleic anhydride was recrystallized from chloroform before use. Enzymatically acetylated radioactive histone H4 was prepared [1] and purified as described previously [3]. Chymotryptic digestion of histone H4, maleylation of chymotryptic histone H4-peptides and tryptic hydrolysis of the peptides was done as described by DeLange et al. [2]. The nomenclature of peptides is that used by these authors. On paper chromatograms fractions were located with ninhydrin spray (1% (w/v) ninhydrin in acetone) on a pilot strip, cut from the margin of the chromatogram. Sections (1 cm) of the pilot strip were oxidized in a sample oxidizer and the radioactivity determined with a liquid scintillation counter. The radioactive peptides were eluted with 30% (w/v) acetic acid from the remainder of the chromatogram and freeze dried.

Sequential degradation of radioactive peptides, identification of amino acid residues and determination of the incorporation of acetyl residues was done as described in a previous paper [1]. The radioactive peptides were sequenced with a 4-fold amount of unfragmented histone H4 as carrier after reaction with sulfonated PITC [4] to prevent losses during sequential degradation. The repetitive yield was 91% and lead to the appearance of radioactive ε-*N*-acetyllysine at each cycle. This background radioactivity amounted at residue 4 to 0.19×10^4 dpm and at residue 12 to 0.3×10^4 dpm for peptide T(M)-2. Acetylation was considered significant if the radioactivity was at least twice of that in the preceding cycle. In the degradation of mono-acetyl T(M)-2 the background radioactivity rose from 0.55×10^3 dpm in residue 1 to 0.93×10^3 dpm in residue 8. From residue 10 onwards no assignments of radioactive amino acids were possible.

3. Results and discussion

Acetylated histone H4 purified via exclusion chromatography [3] consists of subspecies [5] differing in electrophoretic mobility suggesting the existence of mono- and various oligo-acetylated proteins.

The mixture of peptides resulting from chymotryptic degradation of histone H4 separated into two major radioactive fractions (fig.1a). Fraction II after rechromatography gave a peptide with an amino acid

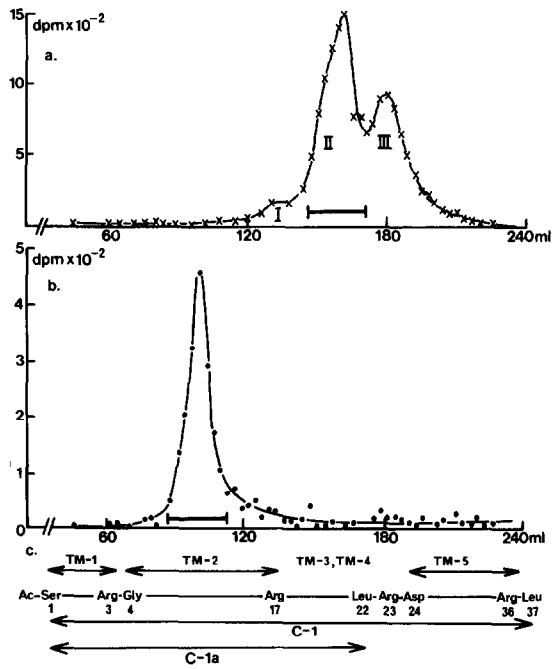

Fig.1. Isolation of histone H4 peptides. 1a: Chymotryptic digest of radioactive acetylated histone H4. (1.7 mg acetylated histone H4 (6.4 X 10^6 dpm), 20 mg carrier histone H4). Peptide mixture in 50% formic acid applied to Sephadex G-75 column (1.5 X 150 cm), equilibrated with 30% (w/v) acetic acid and eluted with the same solvent. Flow rate: 24 ml/h, fraction volume: 3 ml. Fractions I–III were pooled as indicated. 10 μl aliquots of the fraction were used for radioactivity determination in a liquid scintillation counter. 1b: Tryptic digest of maleylated fraction II. 2.1 mg fraction II (peptide C-1) was maleylated, digested with trypsin and after demaleylation [2] applied to a Sephadex G-25 column (1.5 X 140 cm) and eluted as in fig.1a. 5 μl aliquots were used for radioactivity determinations. 1c: Fragmentation pattern of the N-terminus of histone H4 according to DeLange et al. [2].

composition corresponding to fragment C-1 (residues 1–37) which accounts for 55% of the total radioactivity recovered from the column. The remainder of the activity is contained in fraction III corresponding in amino acid composition to chymotryptic fragment C1-a (residues 1–22) (fig.1c). Fraction II (fragment C-1) was maleylated and subsequently digested with trypsin [2] to result in a single radioactive fraction (fig.1b) containing the two peptides T(M)-2 and T(M)-5 (residues 4–17 and 24–37) and 96% of the radioactivity applied to the column (figs.1c and 3). The simultaneously generated peptides T(M)-1 and T(M)-3 do not contain lysine which could undergo acetylation, only peptide T(M)-4 contains lysine which however has been reported to undergo methylation 'in vivo' [2]. The chromatogram (fig.1b) does not give any indication of the presence of a small radioactive peptide resembling T(M)-4. Peptide T(M)-2 contains 4 lysine residues and T(M)-5 one lysine residue. None of the lysine residues coincides in its position with a lysine residue of the other peptide and paper chromatography of the T(M)-4, T(M)-5 mixture showed that the latter was not radioactive (fig.3). Therefore the mixture was sequentially degraded with a repetitive yield of 91% to determine the degree of acetylation of lysine residues. All the radioactivity is accounted for by the acetylation of the lysine residues in peptide T(M)-2 (fig.2).

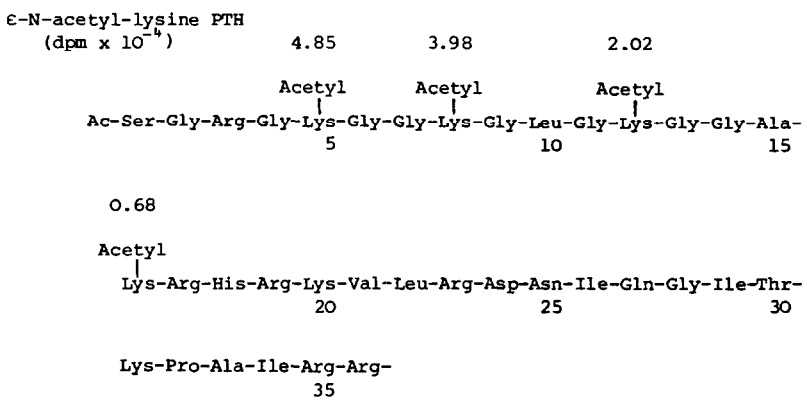

Fig.2. Sites of enzymatic 'in vitro' acetylation of lysine residues in histone H4.

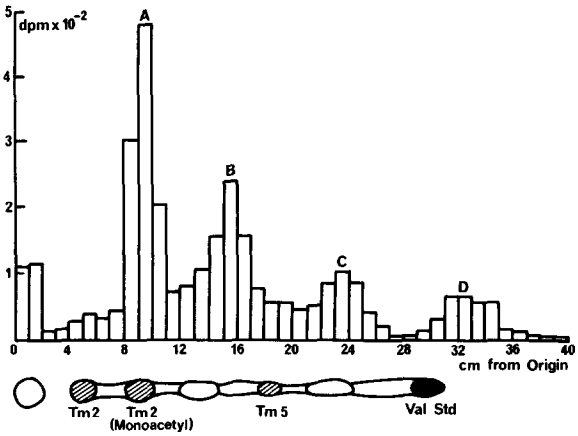

Fig.3. Paper chromatography of peptides T(M)-2 and T(M)-5. Tryptic peptides (fig.1b) were chromatographed in solvent system 2 described by DeLange et al. [8] together with valine standard.

On paper chromatography [8] Fraction II (containing the radioactive peptide T(M)-2 and the non-acetylated peptide T(M)-5) is resolved into four radioactive fractions differing from each other in R_F values by equal increments (fig.3). Fraction A had been shown by DeLange et al. [2] to be mono-acetylated T(M)-2; Fraction B, C, and D probably represent T(M)-2 species with 2,3 and 4 acetyl residues.

Sequential degradation of mono-acetylated T(M)-2 revealed that none of the lysine residues was preferentially acetylated at the exclusion of the other (fig.4) indicating that the sample of mono-acetylated T(M)-2 contains several species of mono-acetylated sequences. This result shows that lysine in mono-acetyl histone H4 has been acetylated by the enzyme either in the 5, 8 or possibly 12 position resulting in different types of mono-acetyl histone H4 indicating that the acetylating enzyme(s) probably recognizes the immediate neighbourhood of a lysine residue and acetylation at these sites occurs independently of each other. A comparison of the major acetylated sites shows that these lysine residues are the same as those acetylated 'in vivo' [9]. They all have a similar environment namely Gly–Lys–X where X may be glycine or alanine [9]. This environment occurs also in one of the acetylation sites of histone H3 (Lys 14) whereas three out of the four remaining acetylation sites in that histone have a different environment namely either serine or threonine immediately precede or follow lysine [1]. These differences in the acetylation sites may well indicate the existence of several acetylating enzymes.

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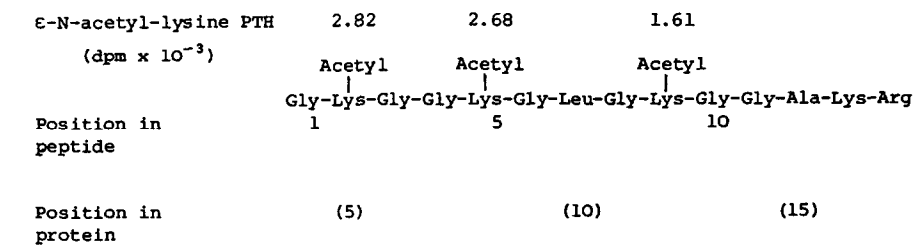

Fig.4. Sites of enzymatic 'in vitro' acetylation of lysine residues in mono-acetylated peptide T(M)-2 (see also fig.3).

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