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PHOTO-INDUCED CROSSLINKING OF HISTONES H3 AND H1 TO DNA IN DEOXYRIBONUCLEOPROTEIN: IMPLICATION IN STUDYING HISTONE-DNA INTERACTIONS

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A thymine-modified derivative of histone H3, isolated as a result of heat treatment of covalently crosslinked DNA-protein photoadduct from UV-irradiated chromatin, was obtained. Sequence analysis of one of its tryptic peptides revealed that lysine-14 of the N-terminal tail of the histone H3 molecule covalently binds to thymine residue of DNA. This type of UV-crosslinking is most probably the only type for histone H3 and, possibly, for H1.

Ultraviolet irradiation of chromatin at λ=254 nm produces, along with other damages, DNA-protein covalent crosslinks [1]. There is growing interest in this problem due to the biological role assigned to DNA-protein crosslinks and also because of the use of UV-crosslinking as a method for studying protein-DNA interactions.

Recently, we have isolated DNA-histone photoadduct from UV-irradiated DNP, which on subsequent heating or acid treatment releases free histones identified as H1 and H3 [2]. In this paper we present evidence that the thymine-lysine photoreaction plays an important role in the photocrosslinking of histones to DNA and that thymine-lysine crosslinking is the only type for H3 and, probably, for H1.

Histone H3 is released from DNA-histone photoadduct by reaction of Saito [3] and contained modified lysine-14 residue in the tryptic peptide Ser_{10}-Lys_{18}.

Abbreviations: DNP, deoxyribonucleoprotein; RP-HPLC, reverse-phase high-pressure liquid chromatography; UV, ultraviolet; SDS, sodium dodecyl sulfate; TFA, trifluoroacetic acid; TLC, thin-layer chromatography.

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These results are direct evidence of existence of an intimate contact between the N-terminal domain of histone H3 and DNA in chromatin structure.

MATERIALS AND METHODS

Preparation of DNP. DNP from calf thymus nuclei was prepared as described by Mandel et al. [4].

Ultraviolet irradiation. DNP (~3 un. A_{260} in 1 mM Tris-HCl buffer, pH 7,8) was irradiated in quartz tubes (d=2 mm) in a flow with a BUV-15 lamp (254 nm) at a dose rate of 0.08 J/m^2 per s (measured with a Black-Ray ultraviolet meter) at 0°C for 30 min.

Isolation of a crosslinked DNA-protein complex. The DNA-protein covalent complex was isolated from irradiated DNP by chromatography on hydroxylapatite [2] or repeated extraction with phenol from 1% SDS [5].

Polyacrylamide gel electrophoresis. Discontinious SDS-electrophoresis was carried out by the method of Laemmli [6], using 15% gel. The gels were stained for 30 min with 0,5% Coomassie blue R-250 in aqueous 30% isopropanol/10% acetic acid for 30 min and destained in aqueous 10% ethanol/5% acetic acid.

Radioactive labeling of histone. Individual stained zones were excised from the gel, washed extensively with 15% methanol and then dried by liophilization. The dehydrated gel slices were added to dissolved Bolton-Hunter reagent (7,4 MBk) in 20-40 µl of anhydrous dioxane and were allowed to absorb reagent solution for about 45 min at room temperature, and then 200 µl of ice-cold 0,1 M sodium borate buffer, pH 8,5 was added. The reaction was continued for 1 h at 0° and at 4°C overnight. The gel slices were washed with 15% methanol and dried by lyophilization.

Peptide mapping. Protein-containing gel slices were digested with thermolysine (50 mg/ml) in 0,5 ml of 0,2 M N-ethylmorpholine-acetic acid, pH 7,8 at 45° C overnight. Following incubation, the buffer containing peptides released was separated from the gel by centrifugation. The gel was then subjected to two N-ethylmorpholine buffer wash-centrifugation cycles. The combined supernatants were lyophilized. Peptide residues were dissolved in 20 µl of pyridine-acetate buffer, pH 3,5. 1-5 µl were applied to 0,1 mm cellulose TLC plates (20x20 cm), which were sprayed with pyridine/acetic acid/water (10:100:890) and subjected to 800 V for 1-1,5 h in a water-cooled thin-layer apparatus (Flat Bed Apparatus FBE 3000, Pharmacia, Sweden) using the same buffer. Ascending chromatography was carried out in the second dimension for 6-7 h in butanol/acetic acid/pyridine/water (15:3:10:12) according to Levenson and Marcus [7]. Plates were autoradiographed for 2-10 h at -20° C using X-ray film and intensifying screen.

Isolation of modified histone H3. The modified histone H3 was isolated from the DNA-protein complex by RP-HPLC [8]. The amino acid composition of the protein after acid hydrolysis (6 N HCl, 105° C, 24 h) was determined on a Hitachi-835 analyzer (Japan) with 2-amino-6(1-thyminyl)hexanoic acid as a marker.

Identification of modified peptide in histone H3. The modified histone H3 (5 mg/ml) was digested with TPCK-trypsin (3% by weight of protein content) in 0,05 M ammonium bicarbonate, pH 8 for 4 h

at 37° C. The tryptic peptides were separated by RP-HPLC on a C_8 Ultrasphere (4,6x250 mm) column using an acetonitrile concentration gradient in 0,1% TFA. Stepwise Edman degradation of the peptide was performed in manual variant [9].

Preparation of 2-amino-6(1-thyminyl)hexanoic acid. 2-Amino-6-(1-thyminyl)hexanoic acid was prepared as described by Saito et al. [3] in the modification of Shetlar [10]. The material was fractionated by RP-HPLC using an "Ultrasphere IP5" column (4,6x x250 mm) at 25°C, eluant 30 mM ammonium formiate, pH 3,85, 1% v/v methanol, and was subjected to TLC analysis on silica gel (150x150 mm) in isopropanol/30% ammonium/water, 7:1:2, R_f=0,5.

Trypsin treatment of irradiated DNP. The irradiated DNP was digested with trypsin as in [11], using trypsin:the total histone ratio 1:250. In the presence of 80 mM NaCl, 0,1 mM EDTA, 10 mM Tris-HCl, pH 7,5 4h trypsinization at 4°C digested all the histones to their enzyme resistant fragments as revealed by electrophoresis. The reaction was terminated by adding a soybean inhibitor. The small tryptic digestion products of histones were removed by extensive dialysis [12]. The material was dissociated by 2M NaCl, 5 M urea and was fractionated on hydroxylapatite.

RESULTS

Participation of histones H3 and H1 in the formation of thermolabile photoadduct induced by UV-irradiation of DNP.

Earlier we have showed that all five fractions of histones are approximately uniformly crosslinked to DNA in DNP [4]. The isolated by chromatography on hydroxylapatite covalent DNA-protein complex (the protein/DNA ratio 0,27 [2]) was subjected to heat treatment (70°C, 2h) and released proteins were separated by SDS/ polyacrylamide gel electrophoresis (Fig.1). Electrophoretic analysis yielded three dominant bands corresponding to the mobility of

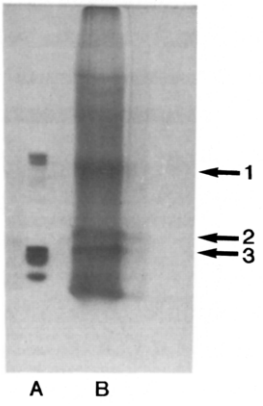

Fig.1. Gel electrophoresis of released proteins from the heat-treated DNA-protein complex (B), A - total histone.

histones H1, H3 and a component X with the mobility lower than that of H3. Other histones are quite faint bands.

We used a two-dimensional thermolysine peptide map analysis of the [^{125}I] Bolton-Hunter reagent labelled material in our modification for identification of the proteins. The peptide maps for the released proteins are comparable favorably with the maps of control histones and therefore help to identify the proteins according to bands 1 and 3 as H1 and H3, respectively.

We estimated that the appearance of X (band 2 in Fig. 1) was related to the procedure of isolation (chromatography on hydroxylapatite or extraction with phenol) of crosslinking proteins from irradiated DNP. By the peptide mapping analysis we have estimated that X is not HMG 17. It represents, most probably, the autolytic product of histone H1.

The participation of thymine-lysine photoaddition in photocrosslinking of H3 to DNA.

A pure fraction of H3 from the heat-treated DNA-protein complex was isolated using HPLC on a C_8 Ultrasphere column and an acetonitrile inverse gradient [8]. The analysis of the acid hydrolysate of isolated H3 revealed the presence of peak between methionine and isoleucine which was identified as 2-amino-6(1-thyminyl)hexanoic acid. This result indicates a possible role of the thymine-lysine photoexchange reaction in the photocrosslinking of histone H3 with DNA in DNP. A mechanism of photoreaction leading to the formation of thymine-lysine crosslink was proposed by Saito [3]. It may be supposed, that the photoexcited state of thymine N(3) monoanion in DNA reacting with neighboring lysine ε-amino group of histone H3 produces DNA-histone adduct, which on subsequent heating releases free histone containing partially modified lysine residue. Other way of the appearance of thymine-lysine photoadduct as a side product of the cytosine-lysine photoreaction has been recently proposed by Shetlar [10].

Our previous work has demonstrated that this type of crosslinking is formed mainly by histones H1 and H3 and it is most probably that the thymine-lysine or cytosine-lysine crosslinking is the only type for H3 [2].

UV-irradiation crosslinks of H1 and H3 to DNA via their tails only.

In the experiments designed to study the involvement of globular regions of histone molecules in photocrosslinking to DNA, irradiated DNP was subjected to controlled digestion with trypsin at 4^{o}C to cleave the basic tails but to preserve crosslinks. Hydroxylapatite chromatography in the presence of dissociating

buffer has been used to separate trypsin-resistant photochemically crosslinked with DNA fragments of histones from free ones. Earlier it was estimated that the electrophoretical patterns of DNP tryptic digestion before and after chromatography on hydroxylapatite were identical under the experimental conditions described lower (data not shown). In order to get modified fragments the column with immobilized DNA and DNA-peptides was heated for 2 h at 70°C and subjected to elution with 2 M NaCl, 5 M urea, 10 mM sodium phosphate, pH 6,8. Electrophoretic analysis of the isolated protein fraction yielded no globular fragments of H3 or H1. Such a finding means that structured central domains of these histone molecules were not crosslinked to DNA, and once cleaved by trypsin, they were stripped from DNP during column fractionation. Therefore the covalent crosslinking of H3 and H1 to DNA in DNP, probably, is accomplished via non-structured basic tails of the molecules only. However, the existance of such an interaction needs a direct proof.

Localization of the lysine residue in histone H3 forming a thymine-lysine crosslink.

The isolated modified H3 was hydrolysed with trypsin and the peptides were fractionated by RP-HPLC (Fig.2). The peptides were detected at 210 and 254 nm. On the chromatogram there are two peaks, absorbing at 254 nm, which can be assigned to peptides,

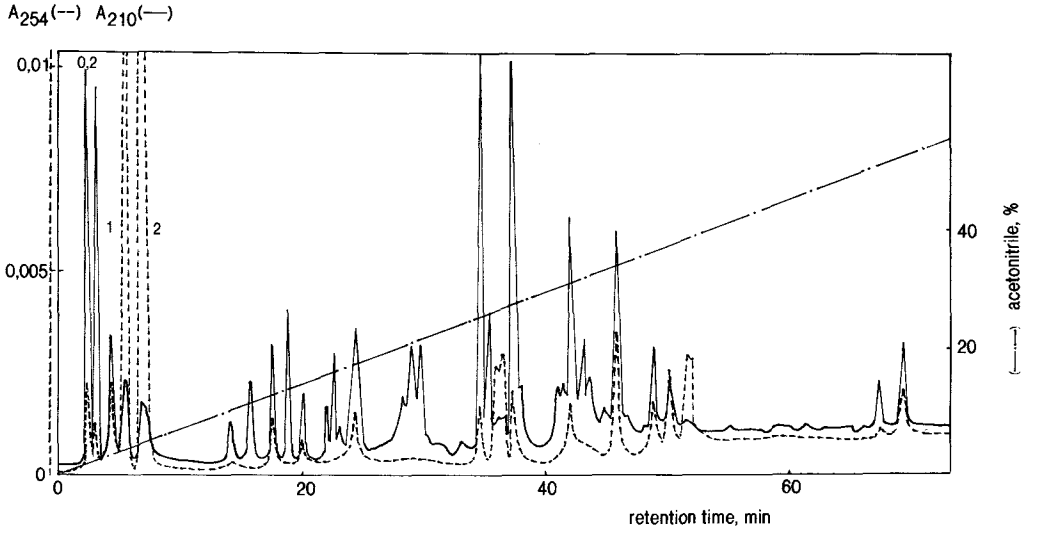

Fig.2. Separation of tryptic peptides of modified histone H3 on a C8 Ultrasphere column (4,6x250mm) using a linear gradient from 0 to 60% acetonitrile in 0,1% TFA at 1 ml/min. Peaks were UV-detected at 210 and 254 nm.

containing a modified lysine residue. Histone H3 itself and its peptides should not significantly absorb light with λ=254 nm [13]. The analysis showed that fraction 1 is a pure peptide while fraction 2 contains a mixture of peptides. We did not succeed in separation this mixture by rechromatography, since low yields were obtained at each step of separation. This led us to use only fraction 1 for sequence analysis. The resultant amino acid sequence of this peptide is STGG. Amino acid analysis of fraction 1 confirmed proposed peptide S_{10}-K_{18} with lysine-14 modified by thymine.

DISCUSSION

In recent years, numerous studies have appeared, indicating that irradiation of deoxyribonucleoproteins with UV-light is accompanied by the formation of covalent protein-DNA crosslinks [1], although the photochemistry has been defined only in a few model systems [10,14]. Both our laboratory and that of Saito have identified thymine-lysine in acid hydrolysate of UV-irradiated DNP [2,3]

In this work we present the results of an investigation of the heat-released protein fraction from UV-crosslinking DNA-protein complex, because, in accordance with the mechanism of Saito reaction this fraction may contain modified histones [3]. It was shown that this type of crosslink is, probably, dominant for histones H3 and H1. Modified H3 was used for localization of a modified lysine residue in the primary protein structure. It was established that lysine-14 of N-terminal tail of the histone H3 is in contact with DNA in DNP. Moreover, we have found that the UV-crosslinking of histone H3, and probably H1, proceeds via the non-structured basic tails of their molecules only. Recently this unexpected property of H3 was confirmed by immunochemical analysis [15]. However, as far as we know, this is our first report estimating the primary structure of the covalently UV-linked peptide of histone H3.

The importance of N-terminal segments of histones in the chromatin structure and function is still not clear [16]. In our experiments two different types of histones - linker histone H1 and core histone H3 - were observed to form the same thymine-lysine covalent links. This finding is a new argument for extensive investigation of relations between H1 and H3. The participance of histone H3 as signal in structural regulation of chromatin by H1 has been recently suggested by Mazen et al. [17].

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