CLUSTERS OF NONHISTONE CHROMOSOMAL PROTEIN HMG1 MOLECULES IN INTACT CHROMATIN

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1. Introduction

Chromatin of eukaryotic cells consists of repeating deoxyribonucleoprotein particles, nucleosomes, each containing ~200 basepairs of DNA and 9 histone molecules (H1, 2 X H2a, 2 X H2b, 2 X H3, 2 X H4) (reviewed [1]). In turn, nucleosomal chains seem to be regularly arranged to form different types of supranucleosomal structures [2,3]. Treatment of chromatin with bifunctional reagents leads to the formation of protein oligomers which due to the existence of both nucleosomal and supranucleosomal levels of chromatin structure can be subdivided into two main groups.

(1) Oligomers of the molecules belonging to one nucleosome, and therefore their structural arrangement and protein composition reflect the intranucleosomal structure.
(2) Oligomers of the proteins belonging to different nucleosomes. Their organization reflects the supranucleosomal structure of chromatin.

The methyl-4-mercaptobutyrimidate mediated polymer of histone H1 isolated and analysed earlier [4] belongs to the second group. It is represented by a series of oligomers consisting of e.g., 12, 24 and 36 molecules of histone H1. This periodicity suggests the existence of repeating 12-nucleosome units in chromatin structure. Poly-H1 was shown to contain a minor component with electrophoretic mobility similar to that of nonhistone chromosomal protein HMG1 [5].

These data indicate that treatment of intact chromatin with bifunctional reagents generates independent homopolymers of histone H1 and nonhistone chromosomal protein HMG1 rather than heteropolymers. Formation of HMG1 oligomers implies the close proximity of adjacent molecules of this nonhistone protein in chromatin of isolated nuclei.

Abbreviations: HMG, high mobility group; Tris, tris-hydroxymethylaminomethane; MMB, methyl-4-mercaptobutyrimidate; SDS, sodium dodecylsulphate

2. Materials and methods

2.1. *Isolation of the initial polymer fraction of nuclear proteins*

Intact cell nuclei were isolated from calf thymus, treated with MMB, and the polymer fraction containing histone H1 and nonhistone protein M1 was obtained as in [4], except that treatment of the isolated protein with iodoacetamide was omitted.

2.2. *Separation of the initial polymer fraction on carboxymethylcellulose*

The polymerized protein (2 mg) was dissolved in 1 ml 2.5 M urea, 20 mM Tris–HCl (pH 7.5) (buffer A) and applied to a 0.5 X 7 cm carboxymethylcellulose CM-52 (Whatman) column equilibrated with 20 mM Tris–HCl (pH 7.5). Elution was done with 9 ml buffer A and then 6 ml 1.5 M NaCl, 20 mM Tris–HCl (pH 7.5) (buffer B). The fractions corresponding to peaks 1 and 2 (fig.1) were collected. The protein of the fractions was precipitated with 15% trichloroacetic acid, centrifuged, and the pellet obtained was washed 3 times with acetone and dried in the open air.

2.3. *Electrophoretic analysis*

To determine the protein composition of the polymer, samples were incubated with 1% β-mercaptoethanol for 18 h prior to electrophoresis. This treatment resulted in the reduction of S–S bridges between MMB residues and the splitting of oligomers into con-

stituent protein molecules which were identified electrophoretically. Electrophoresis was run in 15% polyacrylamide gel with 0.9 M acetic acid and 5 M urea or 0.1% SDS as in [4]. For molecular weight calibration, the following standard proteins (Boehringer) were used: ovalbumin, serum albumin, and DNA-polymerase I from *Escherichia coli*. Partial splitting of protein oligomers was done as in [4].

2.4. *Determination of the N-terminal amino acid*

The N-terminal amino acid was determined by the dansyl method [6]. After dansylation the protein was hydrolysed with 6 M HCl for 18 h at 105°C and then dried. Dansyl amino acid was extracted with acetone and identified by thin-layer chromatography on polyamide plates [7].

2.5. *Standard samples*

Samples of total acid-soluble protein, histone H1 and nonhistone protein HMG1 from calf thymus were prepared as in [4].

3. Results

Treatment of cell nuclei with bifunctional reagents results in the formation of a number of oligomeric complexes of histone molecules. The fraction extracted with 3.5% $HClO_4$ has been shown to contain almost pure histone H1 polymer [4]. A minor protein component of this fraction earlier referred to as M1 [4] was electrophoretically similar to HMG1 (fig.2 in [4]).

Separation of the corresponding initial protein polymer (see section 2) on a carboxymethylcellulose column resulted in obtaining of two polymer fraction (fig.1). Reduction of the two fractions and subsequent electrophoretic analysis showed that fractions I and II (fig.1) contained pure poly(M1) and poly(H1) respectively (fig.2); mobilities of M1 and HMG1 in the acetic acid–urea electrophoretic system being identical. As shown in [4], mobility of M1 in the SDS-containing electrophoretic system was somewhat lower than that of standard HMG1. Here we omitted treatment of the isolated crosslinked proteins with iodoacetamide which could change the electrophoretic mobility. As a result, the mobility of protein M1 from the obtained polymer and that of HMG1 were found to be identical (fig.3).

The N-terminal amino acid of the M1 molecule was shown to be glycine like in HMG1 [8]. The solu-

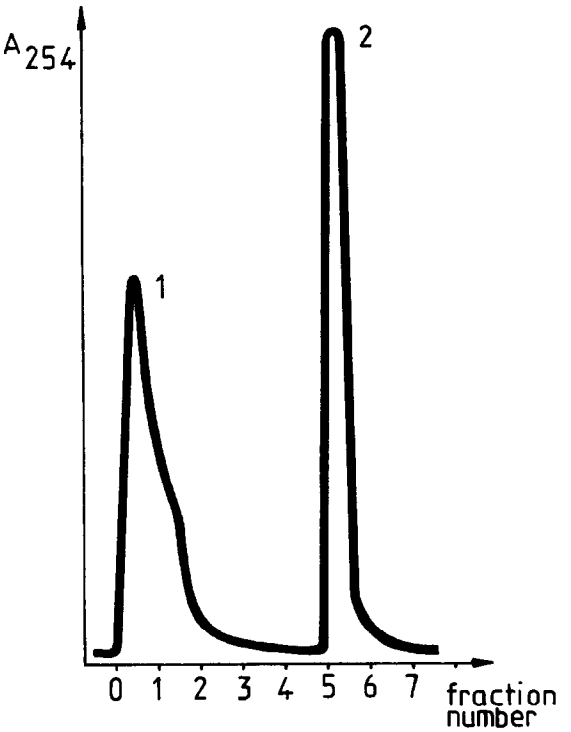

Fig.1. Separation of initial polymer on carboxymethylcellulose CM-52. The sample applied was in buffer A. Elution of fractions 1–5 was performed with buffer A, and fractions 6–8 with buffer B. Each fraction was 2 ml.

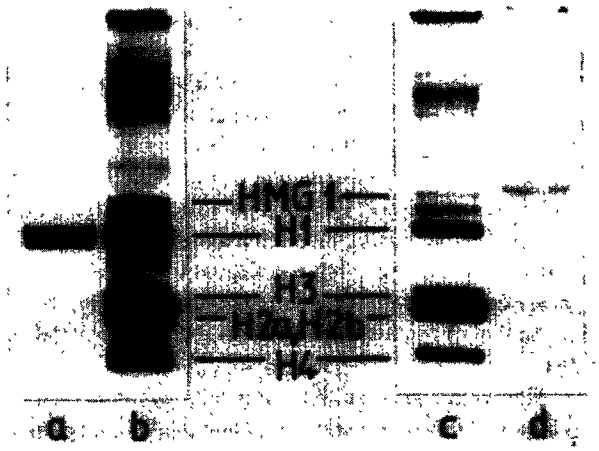

Fig.2. Electrophoresis of protein polymer fractions 1 and 2 obtained upon separation on carboxymethylcellulose (fig.1) in 15% polyacrylamide gel containing acetic acid and urea: (a) reduced polymer fraction 2; (b,c) standard acid soluble protein from calf thymus; (d) reduced protein fraction 1.

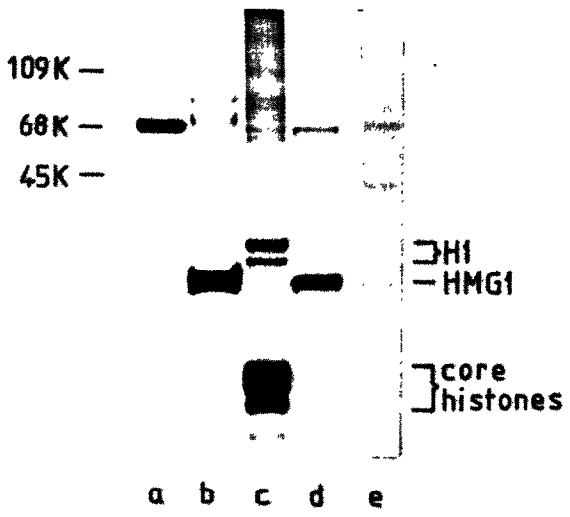

Fig.3. Electrophoresis of protein polymer fraction 1 obtained upon separation on carboxymethylcellulose (fig.1) in 15% polyacrylamide gel containing SDS: (a) initial polymer; (b) reduced polymer; (c) standard acid soluble protein from calf thymus; (d) standard protein HMG1; (e) partially split polymer. Lines (left) show positions of standard proteins, ovalbumin (45 000 M_r); serum albumin (68 000 M_r); DNA-polymerase I from *E. coli* (109 000 M_r).

bilization of both H1 and HMG1 in 3.5% $HClO_4$ (see Methods in [4]), identity of their electrophoretic mobilities in two different systems as well as coincidence of the N-terminal amino acids permit us to conclude that protein M1 is identical with nonhistone chromosomal protein HMG1.

Comparison of electrophoretic mobility of oligo(HMG1) with that of standard proteins showed that this fraction contained homogeneous protein species with app. M_r 68 000 (fig.3). Inasmuch as the electrophoretic mobility of HMG1 corresponds to app. M_r ~24 500 [9], the oligo(HMG1) elicited after treatment of chromatin with the bifunctional reagent is most likely to be a trimer. This suggestion is also supported by the fact that partial splitting of oligo(HMG1) leads to the formation of only one intermediate (dimer) between the initial oligo(HMG1) and mono(HMG1) (fig.3). The somewhat lower app. M_r of the oligomer can be accounted for by partial overlapping of polypeptide chains of the monomer molecules within the oligomer. Elution of HMG1 trimer in the void volume of a Sephadex G-200 column (see Methods in [4]) was likely due to HMG1 binding to poly(H1). Since the binding between HMG1 and histone H1 is of hydrophobic nature [9], their complex can obviously resist acidic conditions of gel filtration. This suggestion is supported by the finding that in the absence of poly(H1) the purified oligo(HMG1) was eluted from a Sephadex G-200 column not in the void volume but according to its actual M_r (not shown).

4. Discussion

Formation of HMG1 trimers after treatment of cell nuclei with the bifunctional reagent strongly suggests that molecules of HMG1 in chromatin are arranged, at least partly, in groups of three, and the adjacent molecules within these groups are closely spaced. It should be noted that besides SH groups of MMB residues those of cysteine residues of the HMGI molecules may also contribute to the formation of these oligomers.

An eukaryotic cell is known to contain ~10^6 molecules nonhistone chromosomal protein HMG1/nucleus [10]. HMG1 has been found in the fraction which constitutes ~9% of the total chromatin and contains apart from the full complement of core histones also nonhistone proteins HMG1 and HMG2 but is devoid of histone H1 [11]. Ratio of core histones to HMG proteins in this fraction was shown to be rather similar to that of core histones to histone H1 in the total chromatin. Both HMG1 and HMG2 were present in mono- and oligonucleosomes. On this basis, the two proteins were supposed to replace histone H1 in nucleosomes [11].

Crosslinking of HMG1 in nuclei into oligomers similar to poly(H1) but shorter in length, as shown here, agrees well with this supposition. HMG1 can therefore influence the formation and transitions of the supranucleosomal chromatin structure like histone H1 [12].

Finally, these data indicate that the discrete structure of poly(H1) [4] is not due to insertion of the minor protein into the polymer chain. It is reasonable to suggest that the sites between the 12-nucleosomal units are characterized by disturbance of contact between the adjacent histone H1 molecules possibly due to anomalously long internucleosomal distances.

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