THE PRESENCE OF HIGH MOBILITY GROUP NON-HISTONE CHROMATIN PROTEINS IN ISOLATED NUCLEOSOMES

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1. Introduction

Recent models of the structure of chromatin suggest that it is composed of repeating subunits, each subunit or nucleosome consisting of about 200 base pairs of DNA associated with the five histones [1,2]. It is thought that 140 base pairs of DNA are associated with an octamer of two each of the four histones H2A, H2B, H3 and H4 [3], whilst H1 is probably bound to the section of DNA linking the subunits [4]. Little is know as regards how the non-histone chromosomal proteins fit into this model and it is not known which, if any, of the non-histone proteins are bound to nucleosomes. Our laboratory is currently studying a particular group of non-histone chromosomal proteins, called the High Mobility Group (HMG) proteins [5], and it was therefore of interest to find out whether these proteins are associated with nucleosomes.

The HMG proteins are a group of proteins loosely bound to the chromatin, being dissociated from it with 0.35 M NaCl [6]. They have some properties in common with histones, e.g., having approximately 25% basic amino acids and are extractable with acids [7]. However, they are present in much smaller quantities than the histones, the total HMG protein being about 3% by weight of the DNA in thymus. Three of the HMG proteins, HMG1, HMG2 and HMG17, have been isolated in a pure form [6] and the complete amino acid sequence of protein HMG17 has recently been determined [8]. In this paper we demonstrate that the HMG proteins are present in nucleosomes which have been isolated by gel-filtration chromatography following nuclease digestion of rabbit thymus nuclei.

2. Materials and methods

Nuclei were prepared from rabbit thymus as follows. Minced thymus (~8 g) was blended in 100 ml of 0.25 M sucrose, 10 mM Tris–HCl (pH 7.5), 10 mM $MgCl_2$, 0.5 mM phenylmethylsulphonyl fluoride (PMSF). (PMSF was dissolved in isopropanol at a concentration of 50 mM just prior to addition to buffers.) The homogenate was centrifuged at 2000 × *g* for 10 min and the pellet resuspended in 40 ml of the 0.25 M sucrose buffer. This was mixed with 2.5 vol. of 2.2 M sucrose, 10 mM Tris–HCl (pH 7.5), 10 mM $MgCl_2$, 0.25 mM PMSF, layered over the 2.2 M sucrose buffer, and centrifuged at 30 000 × *g* for 1 h. The purified nuclei were washed once with digestion buffer (see below) containing 0.5 mM PMSF.

Nucleosomes were prepared from the nuclei by a scaled-up modification of the procedure of Shaw et al. [9]. The nuclei (150 mg DNA) were suspended in 50 ml of digestion buffer (0.3 M sucrose, 5 mM Tris, 1 mM $CaCl_2$, adjusted to pH 7.3 with cacodylic acid). Micrococcal deoxyribonuclease (Worthington) was added (150 units/ml) and digestion carried out for 2.5 min at 37°C. The reaction was stopped by the addition of EDTA to 1.7 mM. Fresh PMSF was added and the solution cooled to 0°C. After centrifuging at 2000 × *g* for 20 min, the supernatant was loaded onto a Bio-Gel A-5 m (100–200 mesh) column (5 × 85 cm) and eluted with 0.7 mM EDTA, 10 mM Tris–cacodylate (pH 8), 0.5 mM PMSF at a flow rate of 1 ml/min. Twenty milliliter fractions were collected. The elution profile is shown in fig.1. The fractions containing the monomer (hatched area, fig.1) were pooled for DNA and protein isolation.

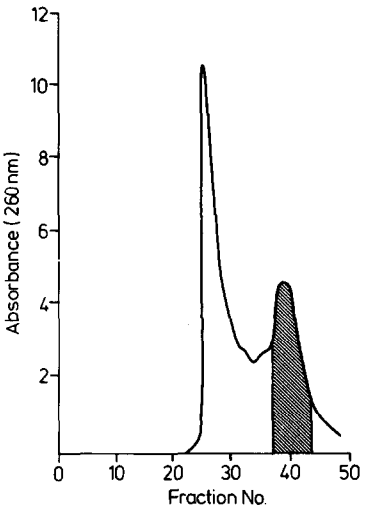

Fig.1. Bio-Gel A-5 m gel filtration chromatography of the nuclease digest. The hatched area represents the fractions containing monomer nucleosomes.

DNA was isolated from the total digest and from the monomer peak essentially as described by Britten et al. [10]. After precipitating the extracted DNA with ethanol, it was washed several times with 70% ethanol to remove salts which may interfere with its electrophoretic migration.

HMG proteins, together with histone H1, were extracted from total digested nuclei and monomer nucleosome by three extractions with 5% perchloric acid [7]. After clarifying the extracts by filtering through glass sinters, the extracts were made 0.3 M with respect to HCl, and six volumes of acetone added. The solutions were left overnight at −20°C before collecting the precipitates by centrifugation. The HMG proteins were separated from the histone Hl by fractional acetone precipitation as follows. The proteins were dissolved in 0.1 N HCl at a concentration of 0.5 mg/ml and three volumes of acetone added to precipitate the bulk of the Hl. After centrifugation, a further three volumes of acetone was added to the supernatant to precipitate HMG proteins which were collected as described above. Prior to electrophoresis, the HMG proteins were reduced with dithiothreitol [11].

Calf thymus total HMG proteins were prepared by the 0.35 M NaCl extraction procedure [6]. Rabbit thymus total HMG proteins were obtained from nuclei by the same method.

SV40 DNA was a generous gift from Dr B. Griffin. This was digested with Hae III endonuclease (Miles) by incubating 10 μg of DNA with 12 units of enzyme for 4 h at 37°C in 10 mM Tris–HCl (pH 7.6), 10 mM $MgCl_2$, 10 mM mercaptoethanol. The cleaved DNA was ethanol-precipitated, washed with 70% ethanol and redissolved in electrophoresis buffer (see below).

Electrophoresis of HMG proteins was carried out using 20% polyacrylamide gels (0.6 × 9 cm) containing 1 M acetic acid as descibed previously [12].

Electrophoresis of DNA was carried out essentially as described by Maniatis et al. [13] using 5% polyacrylamide gel slabs (8 × 8 cm) containing Tris–borate–EDTA (pH 8.3) buffer. Electrophoresis was carried out at 150 V until the bromophenol blue marker reached the bottom of the gel. The gel was stained with ethidium bromide (0.5 μg/ml).

3. Results

The HMG proteins are fairly susceptible to proteolytic degradation and it was for this reason that rabbit thymus was employed in this study, since this tissue appears to have lower levels of proteolytic enzymes than calf thymus. For comparison, gels (a) and (b) of fig.2 show the polyacrylamide gel electrophoresis patterns of calf thymus total HMG proteins and rabbit thymus total HMG proteins, both being prepared by the 0.35 M NaCl extraction procedure [6]. Some histone H1 is also present in both preparations [6]. The rabbit thymus pattern is simpler than that of the calf thymus HMG, the former having only four main proteins–HMG1, HMG2, HMG14 and HMG17 plus a band running ahead of HMG2, which is very variable in quantity and not always present, suggesting that it is a degradation product. Calf thymus total HMG proteins, on the other hand, frequently show many more bands [5]. These extra bands we believe to be degradation products (Goodwin, Walker and Johns, manuscript in preparation). In summary, at present we believe that there are basically only four main HMG proteins in thymus (discounting the microheterogeneity of HMG2 [14]) namely, HMG1, HMG2, HMG14 and HMG17.

In order to be able to rapidly isolate the total HMG proteins from the dilute solutions of monomer nucleosomes obtained by gel filtration (fig.1), it was

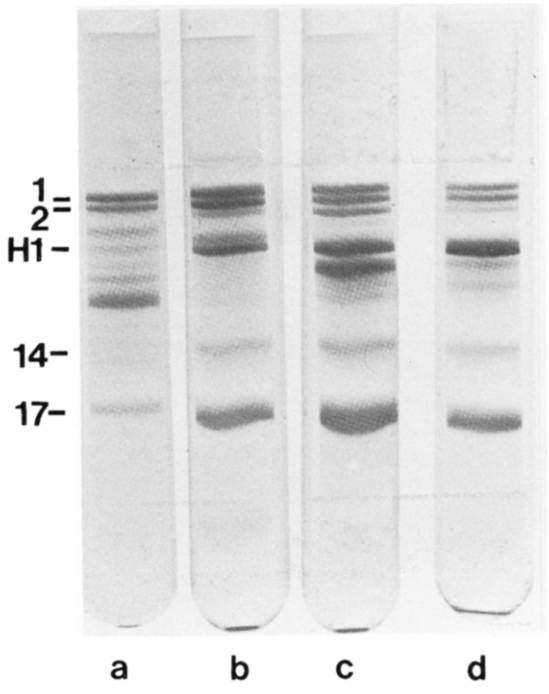

Fig.2. Polyacrylamide gel electrophoresis of HMG proteins: (a) Calf thymus HMG proteins, (b) rabbit thymus HMG proteins, (c) monomer nucleosome HMG proteins, (d) HMG proteins from nuclease digested nuclei.

necessary to employ a different procedure to the usual 0.35 M NaCl extraction method. Since the HMG proteins are soluble in 5% perchloric acid, they were extracted together with histone H1 with this reagent and the total protein precipitated with acetone, the H1 acting as a carrier for bringing down the smaller quantities of HMG proteins. Histone H1 was then removed by fractional acetone precipitation. The HMG proteins obtained in this way from monomer nucleosomes and from the total nuclear digest are shown in gels (c) and (d) of fig.2. As can be seen, proteins HMG1, 2, 14 and 17 are all present in the monomer. Some protein degradation still appears to have occurred despite the presence of PMSF in all the buffers, since bands running just ahead of HMG2 and histone H1 are present also. From a comparison of the staining intensity of gels (b) and (c) of fig.2, an approximate estimate of the quantity of total HMG protein (i.e. HMG1, 2, 14 and 17) in the monomer nucleosome can be made. Gel (c) has about the same amount of HMG proteins as gel (b) which had 50 μg of rabbit thymus total HMG protein loaded. The quantity of protein loaded onto gel (c) was derived from one twelfth of the perchloric acid extracted material from the monomer peak. 3.6 mg of perchloric acid extractable material was obtained from monomer nucleosomes containing 19 mg of DNA. From these figures it can be calculated that the weight of total HMG proteins is about 3% of the DNA in the monomer peak. This figure corresponds to the amount of total HMG proteins which can be isolated from whole thymus nuclei.

The size of the DNA in the monomer peak was determined by polyacrylamide gel electrophoresis (fig.3). A logarithmic plot of the sizes of the SV40 DNA Hae III fragments [15] versus migration distance, gave a straight line from which the size of the monomer DNA could be estimated. A value of 150–160 base pairs was obtained. This is smaller than the 200 base pair repeat, i.e., 40–50 base pairs have been removed during digestion (approximately 10% of the DNA was rendered acid-soluble during digestion). All five histones are present on the monomer (not shown) although histone

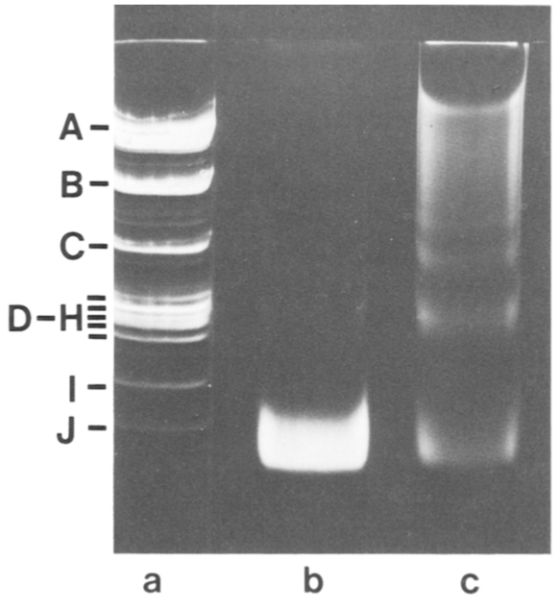

Fig.3. Polyacrylamide gel electrophoresis of DNA: (a) SV40 DNA Hae III digestion products (A = 1600; B = 830; C = 540; D = 370; E = 350; F = 330; G = 320; H = 320; I = 230; J = 170 base pairs), (b) DNA from monomer nucleosomes, (c) DNA from nuclease digested nuclei.

H1 is reduced in quantity; presumably some H1 is lost as the DNA is reduced from 200 to 150–160 base pairs [4].

4. Discussion

It appears from the evidence presented in this paper that the four HMG proteins, HMG1, 2, 14, and 17, are isolated together with monomer nucleosomes. Augenlicht and Lipkin [16] have reported that there is very little non-histone protein in monomers, but they were looking at the total chromosomal protein on SDS polyacryamide gels and they may well not have seen the HMG proteins since they migrate in the histone region and could have been hidden by the 30-fold excess of histone. The fact that the HMG proteins have been identified in isolated nucleosomes would suggest that they are present in this structure in vivo. It is not known if rearrangement of the HMG non-histone proteins takes place under the conditions of nucleosome isolation and it therefore remains to be unequivocally proved that they are part of the in vivo protein complement of nucleosomes. However, there is some evidence to support an association between HMG proteins and histones and this comes from recent studies on the interaction of protein HMG1 with histone H1 [17,18]. The apparent quantitative recovery of HMG proteins from the isolated nucleosomes would also argue against a major rearrangement of chromosomal proteins.

If it is true that HMG proteins are associated with nucleosomes in vivo, it does imply a heterogeneity in the nucleosomes since there is not enough total HMG protein in the nucleus for all the nucleosomes to have HMG proteins bound. It may well be that it is the subpopulation of nucleosomes with HMG proteins bound that are associated with genes that are being actively transcribed [19].

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