Vol. 98, No. 1, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 15, 1981 Pages 330-336

NUCLEOSOME CORES CONTAINING H2B, H3, H4 AND HMG2 ARE RECONSTITUTED

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Received November 26,1980

SUMMARY Nucleosome cores mixed with the high mobility group proteins, HMG1 and HMG2, in 2 M NaCl, 5 M urea, 0.2 mM EDTA and 10 mM Tris pH 7.0, have been reconstituted by salt gradient dialysis. The reconstituted material, in 10 mM Tris pH 7.0, had a sedimentation peak at the same position as that of control nucleosome cores in sucrose density gradient ultracentrifugation. The SDS polyacrylamide gel electrophoresis of the reconstituted nucleosome cores demonstrated that they contain H2B, H3, H4 and HMG2 and are selectively deficient in H2A. The circular dichroism of DNA of the reconstituted cores was indistinguishable from that of control nucleosome cores. The results suggest that HMG2 replaces H2A as a component of the nucleosome histone core during reconstitution.

The high mobility group(HMG) proteins are a group of nonhistone chromosomal proteins. They consist of four main proteins, HMG1, HMG2, HMG14 and HMG17 (1).

HMG14 and HMG17 can be found associated with nucleosome core particles isolated from nuclei (2), and two molecules of HMG14 or HMG17 can be bound to each isolated nucleosome core particle (3-5). Recent reports have shown that HMG14 and HMG17 are involved in maintaining the DNase I sensitive conformation of transcriptionally active regions of genome(6,7). HMG1 and HMG2 are also found associated with nucleosomes isolated from nuclei or chromatin (2,8,9). Both HMG1 and HMG2 exhibit selective affinity for single-stranded DNA (10,11), or interact with histone H1 (12-14). In addition, it

[1] Abbreviations used: HMG, high mobility group; SDS, sodium dodecyl sulfate.

0006-291X/81/010330-07$01.00/0

has recently been shown that HMG2 levels parallel the proliferative activity of individual organs or tissues (15).

In this paper we have reconstituted nucleosome cores in the presence of HMG1 and HMG2 to investigate the binding mechanisms of the HMGs to cores.

MATERIALS AND METHODS

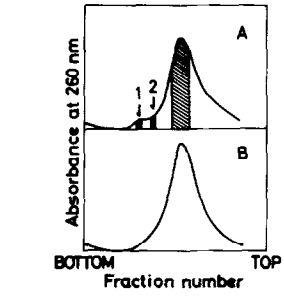
Preparation of HMG1 and HMG2 : Proteins HMG1 and HMG2 were prepared from calf thymus according to the method as described in (16). Mixtures of histone H1 and HMG proteins were extracted with 5% perchloric acid. Histone H1 was eliminated by an overnight precipitation at -20°C with 3 volumes of acetone. After the precipitation was pelleted, the HMGs in the supernatant were precipitated overnight at -20°C by the addition of a further 3 volumes of acetone. The resulting precipitated HMGs were dissolved in 7 mM borate buffer containing 0.1 M NaCl and adjusted to pH 9.0 by NaOH. The solution was then loaded on a CM Sephadex C25 column and eluted by a linear salt gradient of 0.1-1.0 M NaCl in 7 mM borate buffer pH 9.0. The peak fractions corresponding to HMG1 and HMG2 were separately collected and precipitated by acetone, followed by lyophilization. Purities of the HMGs were checked by SDS polyacrylamide gel electrophoresis.

Preparation of nucleosome core particles : Nucleosome cores were prepared from chicken erythrocytes as described in (17). H1-depleted chromatin by 0.65 M NaCl was digested by micrococcal nuclease. Core particles were isolated from chromatin digests by a 5-20% linear sucrose density gradient ultracentrifugation.

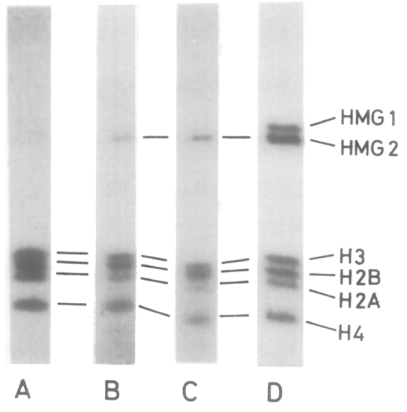
Reconstitution of nucleosome cores in the presence of HMGs : Nucleosome cores were mixed with HMG proteins, HMG1 and HMG2 approximately in equimolar, at various [added HMG(1+2)/nucleosome] (w/w) ratios ranging from 0 to 0.5, in 2 M NaCl, 5 M urea, 0.2 mM EDTA and 10 mM Tris pH 7.0. The final DNA concentration in the mixture was approximately 0.5 mg/ml. Association was accomplished by step-gradient dialysis for 4-6 hr against 2.0, 1.4, 1.0, 0.6, 0.3, 0.1 and 0 M NaCl in sequence, each containing 5 M urea, 0.2 mM EDTA and 10 mM Tris pH 7.0. This was followed by an exhaustive dialysis against 10 mM Tris pH 7.0 to remove urea. As a control, nucleosome cores were reconstituted under the same experimental conditions in the absence of HMGs. Spectropor dialysis tubing was used throughout. Thus reconstituted nucleosome cores were layered onto linear 5-20% sucrose gradients containing 10 mM Tris pH 7.0, then centrifuged in an International SB-283 rotor for 10 hr at 40000 rpm at 4°C, followed by fractionation.

Polyacrylamide gel electrophoresis : Proteins were separated on 15% polyacrylamide gels in 0.1% SDS as described by Laemmli (18). The gels were stained in 0.2% Coomassie blue and destained in 10% acetic acid.

Circular dichroism : Circular dichroism measurements were carried out with a Jasco J-20 spectropolarimeter in temperature controlled (20°C) quartz cells.



(1)



(2)

Figure 1. Sucrose density gradient sedimentation patterns of nucleosome cores reconstituted in the presence and absence of HMG1 and HMG2. (A) Nucleosome cores reconstituted in the presence of HMG1 and HMG2. [added HMG(1+2)/nucleosome](w/w) =0.375. [HMG1]≒[HMG2]. The hatched sedimentation peak fractions were isolated as reconstituted nucleosome cores. (B) Control nucleosome cores reconstituted in the absence of HMGs. Each sample in 10 mM Tris pH 7.0 was loaded on a 5-20% linear sucrose gradient containing 10 mM Tris pH 7.0. Centrifugation was in an International SB-283 rotor at 40000 rpm and 4°C for 10 hr.

Figure 2. SDS polyacrylamide gel electrophoresis of reconstituted nucleosome cores. (A) Control nucleosome cores : [added HMG(1+2)/nucleosome](w/w)=0. (B) [added HMG(1+2)/nucleosome](w/w)=0.25. (C) [added HMG(1+2)/nucleosome](w/w)=0.375. Samples of slots (A)-(C) were taken from peak fractions of sucrose gradient sedimentation pattern of each reconstituted samples. (D) Sample at [added HMG(1+2)/nucleosome](w/w)=0.375, in 10 mM Tris pH 7.0, after reconstitution but without fractionation by sucrose gradient centrifugation.

RESULTS

Nucleosome cores mixed with varying amounts of HMG1 and HMG2, approximately in equimolar, were reconstituted. The reconstituted samples were run on a 5-20% linear sucrose gradient. As shown in Fig.1, the reconstituted HMG(1+2)/nucleosome sample (HMG/nucleosome =0.375) has a sedimentation peak at the same position as that of the control nucleosome cores reconstituted in the absence of HMGs. Similar sedimentation patterns were obtained for the reconstituted samples at other HMG/nucleosome ratios. The sedimentation peak fractions were isolated as reconstituted nucleosome cores and used in the following experiments.

Fig.2 shows the SDS polyacrylamide gel electrophoresis of reconstituted nucleosome cores (HMG/nucleosome=0.25 and 0.375).

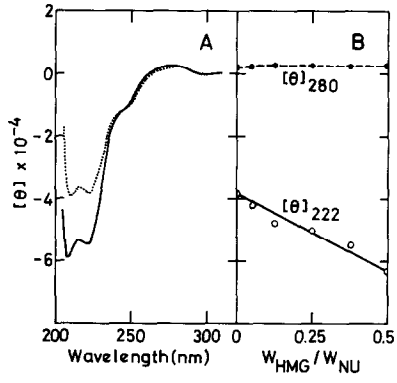
The control nucleosome cores exhibit four core histone bands in equal intensity. It is noteworthy that, in reconstituted nucleosome cores HMG2, but not HMG1, band can be detected and H2A band is selectively faint. The facts suggest a possible replacement of H2A by HMG2 as a component of the histone core during reconstitution. The weak H2A band would be due to coexistence of normally reconstituted cores which have four core histones and no HMGs as components.

As shown in Fig.3A, the circular dichroism spectrum above 250 nm of reconstituted particles (HMG/nucleosome=0.375) is very similar to the spectrum of the control nucleosome cores, indicating that the DNA conformation of the particles is the same as that of normal cores. That this is true also for other reconstituted particles is shown by the plot in Fig.3B, i.e., the ellipticity at 280 nm($[\theta]_{280}$) of the particles remains almost unchanged with HMG/nucleosome ratio. The linear increase of $-[\theta]_{222}$ with HMG/nucleosome ratio indicates the linear increase in the α-helix content in the reconstituted core particles as a function of the amount of added HMGs in the reconstitution.

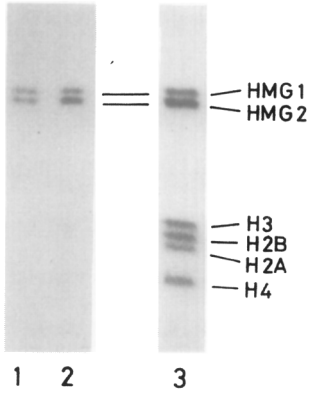
Fig.4 presents the SDS polyacrylamide gel electrophoresis of the fastest moving fractions in the sucrose density gradient sedimentation pattern in Fig.1A. As clearly shown in Fig.4, these fractions contain only HMG1 and HMG2 and are completely deficient in all the core histones. These particles would be the same nucleosome-like particles as those reconstituted from DNA-HMG(1+2) mixtures by Mathis et al. (19).

DISCUSSION

It has been suggested by the present study that HMG2 replaces H2A as a component of the nucleosome histone core during reconstitution. Any major structural difference was not observed between the HMG2-containing core particles and the control nucleosome cores



(3)



(4)

Figure 3. (A) CD spectra of nucleosome cores reconstituted in the absence and presence of HMG1 and HMG2 in 10 mM Tris pH 7.0. -----,Control nucleosome cores; [added HMG(1+2)/nucleosome](w/w)=0. ———,[added HMG(1+2)/nucleosome](w/w)= 0.375. (B) Changes of molar ellipticities at 222 nm or 280 nm of reconstituted nucleosome cores with [added HMG(1+2)/nucleosome](w/w) ratio. Buffer : 10 mM Tris pH 7.0.

Figure 4. Slots 1 and 2 : SDS polyacrylamide gel electrophoresis of the fastest moving fractions, 1 and 2, respectively, in the sucrose density gradient sedimentation pattern in Fig.1A. Slot 3 : Sample at [added HMG(1+2)/nucleosome](w/w) =0.375, in 10 mM Tris pH 7.0, after reconstitution but without fractionation by sucrose gradient centrifugation.

either by the sedimentation behaviors in sucrose density gradient ultracentrifugation or by the circular dichroism of the DNA, except that the former had higher helical contents than the latter.

The interactions between H2A and other core histones are relatively weak (20) and H2A and H2B can be eluted from chromatin at lower salt concentration than H3 and H4 (21). In the presence of 6 M urea, H2A and H2B are depleted from nucleosome cores at a salt concentration as low as 0.2 M NaCl (22). These facts support the possibility that HMG2 might begin to participate in the core particle formation as a substitute for H2A at lower salt concentrations (e.g. $\lesssim$ 0.35 M NaCl) during the present reconstitution procedure in the presence of 5 M urea. On the other hand, the molecular weight of HMG2, 26000 (23), approximates that of the sum of two H2A molecules. HMG2, free in solution, has substantial helical regions

(24,25) as well as the histone core which contains about 50% of α-helix (26). These structural properties of HMG2 appear to provide favorable conditions for a replacement of H2A with HMG2 as a component of the nucleosome histone core. The molecular details of reconstituted nucleosome cores containing H2B, H3, H4 and HMG2 require further investigations.

Some difference has been found between HMG1 and HMG2, although their amino acid compositions (27), molecular weights (23,28) and secondary structures (24,25) are similar each other and they both exhibit selective affinity for single-stranded DNA (10,11). HMG1 binds strongly to certain H1 histone subfractions, whereas HMG2 binds only weakly to H1 histone (12-14). Recently, Seyedin and Kistler (15) have reported that HMG2 levels parallel the proliferative activity of individual organs or tissues, while HMG1 levels do not show marked organ variability, suggesting a role of HMG2 in cell replication.

Both HMG1 and HMG2 have been found associated with nucleosomes isolated from nuclei or chromatin (2,8,9). However, at present, it remains an open question as to whether such novel nucleosome cores as proposed here exist in chromatin. It is tempting to speculate that the novel nucleosomes may be relevant to the chromatin structure in vivo which is prerequisite to replication or transcription.

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