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The chaperone like function of the nonhistone protein HMGB1

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ABSTRACT

Almost all essential nuclear processes as replication, repair, transcription and recombination require the chromatin template to be correctly unwound and than repackaged. The major strategy that the cell uses to overcome the nucleosome barrier is the proper removal of the histone octamer and subsequent deposition onto DNA. Important factors in this multi step phenomenon are the histone chaperones that can assemble nucleosome arrays in vitro in the absence of ATP. The nonhistone protein HMGB1 is a good candidate for a chaperone as its molecule consists of two DNA binding motives, Box's A and B, and a long nonstructured C tail highly negatively charged. HMGB1 protein is known as a nuclear "architectural" factor for its property to bind preferentially to distorted DNA structures and was reported to kink the double helix. Our experiments show that in the classical stepwise dialysis method for nucleosome assembly the addition of HMGB1 protein stimulates more than two times the formation of middle-positioned nucleosomes. The stimulation effect persists in dialysis free experiment when the reconstitution is possible only in the presence of a chaperone. The addition of HMGB1 protein strongly enhanced the formation of a nucleosome in a dose dependant manner. Our results show that the target of HMGB1 action as a chaperone is the DNA fragment not the histone octamer. One possible explanation for the stimulating effect of HMGB1 is the "architectural" property of the protein to associate with the middle of the DNA fragment and to kink it. The acquired V shaped DNA structure is probably conformationals more favorable to wrap around the prefolded histone octamer.

We tested also the role of the post-synthetic acetylation for the chaperone function of HMGB1 protein. The presence of an acetyl groups at Lys 2 decreases strongly the stimulating effect of the protein in the stepwise salt dialysis experiment and the same tendency persisted in the dialysis free experiment.

1. Introduction

The term "chaperone" was first introduced in 1978 by Laskey [1] regarding a nuclear protein purified from the eggs of *Xenopus laevis* that prevented the formation of histones/DNA aggregates. The concept developed and included proteins that facilitated the proper formation of the nucleosome. The assembly as well as the disassembly of the core histones onto DNA is a stepwise process [2]. H2A/H2B dimers are deposited onto the DNA after H3/H4 has been associated with the DNA molecule and the higher order chromatin structures are further stabilized by the linker histones. Conversely, during chromatin disassembly, H2A/H2B dimers are removed prior to removal of H3/H4 from the DNA. The main task of the histone chaperones is to shield the nonspecific interactions between the negatively charged DNA molecule and the positively charged histones and in this way to allow the ordered formation of the nucleosome structure. That is why the histone chaperones are a diverse group of proteins with little sequence similarity but sharing the common feature of being acidic. Another important characteristic of the chaperone proteins is that they are not present in the final nucleosomal structure. At present the function of histone chaperones is considered to be closely related to the action of ATP-dependent chromatin remodeling machines that use the energy provided by ATP hydrolysis in order to break histone-DNA contacts during chromatin disassembly and to reposition histones during chromatin assembly [3]. Almost all essential nuclear processes as replication, repair, transcription and recombination require the chromatin template to be correctly unwound and than repackaged [4]. Therefore the detailed mechanism of the chaperones' function is of considerable interest.

The nonhistone protein HMGB1 (High Mobility Group Box 1) is a good candidate for a chaperone as its molecule consists of two DNA binding motives, Box's A and B, and a long nonstructured C tail highly negatively charged. HMGB1 protein is known as a nuclear "architectural" factor for its property to bind preferentially to distorted DNA structures such as four way junctions [5], cisplatin- and UV-damaged DNA [6–8], and semicatenated DNA loops [9]. HMGB1 was reported to kink the double helix [10–12] and to constrain negative supercoils in plasmid DNA [13–15]. In vivo

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modified protein, isolated from butyrate-treated cells and shown to be monoacetylated at Lys 2 [16], demonstrated an enhanced binding affinity to distorted DNA [16], inability to bend DNA and, instead, an increased capacity to stimulate joining of DNA fragments via their ends [17].

We studied the potential of HMGB1 protein to facilitate nucleosome formation in a dialysis free experiments and the effect of the post synthetic acetylation on the nucleosome reconstitution.

2. Materials and methods

2.1. Preparation of DNA

The 255 bp fragment, containing the 601-nucleosome positioning sequence at the middle, was obtained by polymerase chain reaction (PCR) amplification from plasmid pGem-3Z-601 using primers: forward 5′-GCTCGGAATTCTATCCGACTGGCACCGGCAAG-3′ and reverse 5′-GCATGATTCTTAAGACCGAGTTCATCCCTTATGTG-3′ [18].

2.2. Preparation of proteins

To prepare core histones, T7-based pET3a expression plasmids carrying the cDNA for *X. laevis* full-length core histones H2A, H2B, H3, and H4 were propagated in *Escherichia coli* B121, expressed, and purified as described previously [19]. HMGB1 proteins, either unmodified or in vivo acetylated, were isolated by a nondenaturing salt extraction procedure from Guerin ascites tumor cells grown in the absence and presence of butyrate, respectively, as described previously [16].

2.3. Nucleosome reconstitution

To reconstitute nucleosomes an equimolar mixture of the four histones was dialyzed overnight at 4 °C against histone folding buffer (10 mM Tris, pH 7.5, 5 mM β-mercaptoethanol, 1 mM EDTA) containing 2.0 M NaCl. Histones were then mixed at 1:1 molar ratio with the appropriate DNA consisting of ^{32}P-labeled fragments (50 ng) and cold DNA of appropriate length, plus nonacetylated and acetylated HMGB1 protein samples at 2 M excess, and stepwise dialyzed against decreasing concentration of NaCl down to 10 mM and analyzed on 5% native gel in 0.25× TBE, run at 4 °C. Gels were dried, exposed on Phosphor-Imager screens, and quantified using ImageQuant (molecular dynamics).

Assay of histone deposition in dialysis free experiments in the presence of appropriate HMGB1 protein samples was performed using histone octamers (80 ng/μl) preassembled in 2 M NaCl. Histones were mixed or not (control) with 2 M excess (in respect to octamers) of HMGB1 protein samples and 100 ng of labeled DNA fragments as indicated, incubated for 3 h at room temperature and analyzed on 5% native gel 0.25× TBE, run at 4 °C. Gels were dried, exposed on Phosphor-Imager screens, and quantified using ImageQuant (molecular dynamics).

3. Results

3.1. Reconstitution of nucleosomes in the presence of HMGB1 protein

We applied the classical stepwise salt dialysis method for the depositioning of core histones onto DNA. Equimolar amounts of recombinant core histones purified to homogeneity (Fig. 1) were first mixed in folding buffer and then added to ^{32}P end-labeled DNA fragment containing the 601-nucleosome positioning sequence at the middle of the fragment: 255 bp for centrally positioned nucleosomes. The same experiment was carried out in the presence of native HMGB1, both nonmodified and acetylated at Lys 2 [16], isolated in non-denaturating conditions in the absence or presence of butyrate. As seen in Fig. 2 the non-acetylated protein strongly enhanced the formation of nucleosome particles (Fig 2, panel A,

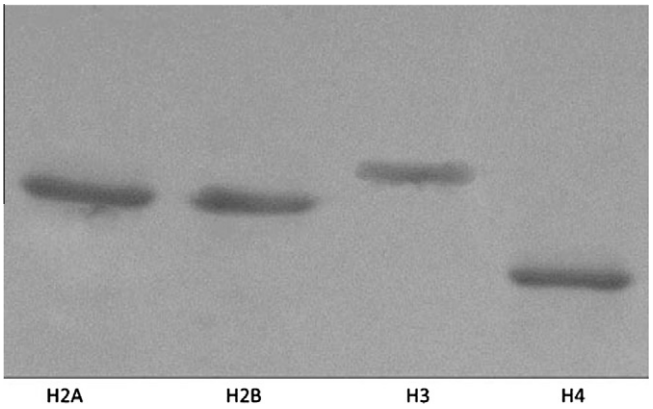

Fig. 1. Electrophoretic analysis of recombinant core histones. The protein samples were run on an 18% polyacrylamide gel containing SDS and stained with Coumassie blue. Lanes 1–4: H2A, H2B, H3 and H4.

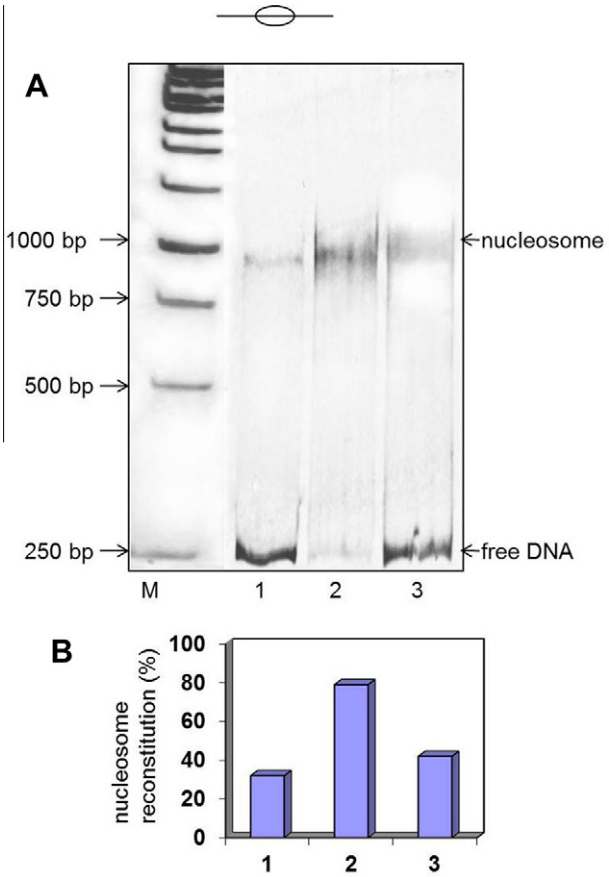

Fig. 2. Nucleosome reconstitution by decreasing salt dialysis in the presence of HMGB1 protein. Panel A: prefolded core histones were reconstituted with labeled ^{32}P DNA fragments containing strong nucleosome positioning sequence by decreasing salt dialysis method and analyzed on 5% polyacrylamide gel. Lane 1: DNA reconstituted with recombinant histones; lane 2: DNA reconstituted with recombinant histones in the presence of 2 M excess of HMGB1 protein; lane 3: DNA reconstituted with recombinant histones in the presence of 2 M excess of acHMGB1 protein, M: DNA molecular marker. Panel B: presents the data from gel-based quantification assay.

compare lanes 1 and 2) while the insertion of an acetyl group at position 2 practically abolished the stimulation effect (Fig 2, panel A, compare lanes 1 and 3).

3.2. Deposition of histones in dialysis free experiments in the presence of HMGB1 proteins

We tested the effect of HMGB1 protein on the assembly of nucleosome particle under experimental conditions when the reconstitution was possible only in the presence of a chaperone [20]. The results presented in Fig. 3 were obtained with the DNA fragment bearing middle-positioned nucleosome sequence. If the prefolded equimolar amounts of recombinant core histones were mixed with a radioactive DNA fragment and incubated at room temperature for 3 h no nucleoprotein particle was detected (Fig. 3, panel A, lane 2). In the presence of 2 or 4 M excess of non acetylated HMGB1 it is clearly seen the formation of a nucleosome structure and the stimulation effect is in a dose dependent manner (Fig. 3, panel A, lanes 4 and 5). The signal is quantified and presented on Fig. 3, panel B. The incubation of HMGB1 with the DNA fragment under the same experimental conditions gave no signal (Fig. 2, lane 3) and served as a control that the radioactive band with higher electrophoretic mobility was due to nucleosome particle but not to HMGB1/DNA complex. The acetylated form of HMGB1 also facilitated the nucleosome assembly but the effect was much weaker (Fig. 3, panel A, lanes 6 and 7; see also panel B) and followed the tendency observed when the salt dialysis

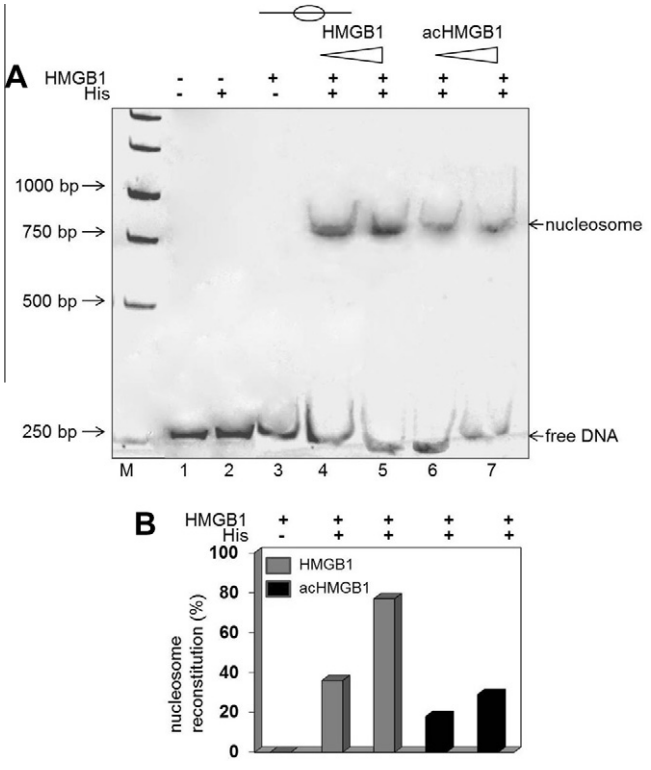

Fig. 3. Nucleosome reconstitution by dialysis free method in the presence of HMGB1 protein. Panel A: prefolded core histones were reconstituted with labeled ^{32}P DNA fragments containing strong nucleosome positioning sequence in dialysis free experiments and analyzed on 5% polyacrylamide gel. Lane 1: free DNA; lane 2: DNA incubated with HMGB1 protein; lane 3: DNA incubated with prefolded core histones; lane 4 and 5: DNA incubated with prefolded core histones in the presence of 2 and 4 M excess of HMGB1 protein, respectively; lanes 5 and 6: DNA incubated with prefolded core histones in the presence of 2 and 4 M excess of acetylated HMGB1 protein (acHMGB1), respectively; M: DNA molecular marker. Panel B: presents the data from gel-based quantification assay.

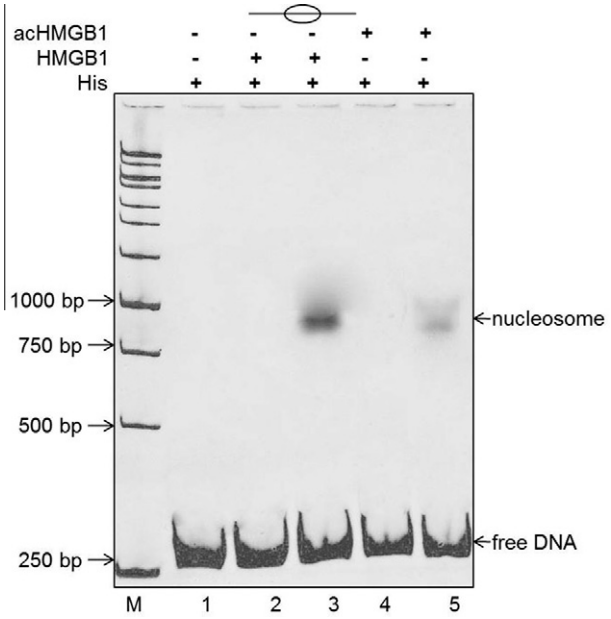

Fig. 4. Deposition of histones in dialysis free experiments in the presence of HMGB1 protein. Panel A: five presents polyacrylamide gel of reconstituted prefolded core histones with labeled ^{32}P DNA fragments containing strong nucleosome positioning sequence performed in dialysis free experiments. Lane 1: free DNA; lane 2 and lane 4: the nonacetylated or acetylated form of HMGB1 protein, respectively, was preincubated with the prefolded core histones for 45 min. at room temperature and than the labeled DNA fragment was added and incubated for additional 3 h; lane 3 and 5: the nonacetylated or acetylated form of HMGB1 protein, respectively, was preincubated with the labeled DNA fragment for 45 min. at room temperature and than the prefolded core histones were added and incubated for additional 3 h; M: DNA molecular marker.

method was applied i.e., the positive effect of HMGB1 decreased upon acetylation.

3.3. The chaperone function of HMGB1 is accomplished through HMGB1/DNA interaction

We tested two possible mechanisms for the chaperone function of HMGB1 protein. In the first case we preincubated HMGB1 protein with the prefolded core histones for 45 min. at room temperature and than the labeled DNA fragment was added and incubated for additional 3 h. In the second case the HMGB1 was preincubated with the DNA under the same experimental conditions and than the mixture was supplemented with the prefolded core histones. The results are presented in Fig. 4. No nucleosome particle was reconstituted when the protein was first mixed with the histones (Fig. 4, lane 2). A strong signal corresponding to a nucleosome structure was detected upon pre-incubation of HMGB1 with the DNA fragment bearing middle-positioned nucleosome sequence (Fig. 4, lane 3). The same experiment was carried out with the acetylated form of HMGB1. The results were similar, the nucleosome was formed only if the protein was first incubated with the DNA (Fig. 4, lane 5), but again the stimulation effect of the acetylated HMGB1 was weaker compared to the non-acetylated form of the protein (Fig. 4 compare lane 3 with lane 5).

4. Discussion

DNA in the eukaryotic nucleus is packed into a nucleoprotein complex termed chromatin with a basic unit, the nucleosome, consisting of an octamer of core histones (two each of H2A, H2B, H3, and H4), around which 1.75 turns of DNA are wrapped [21,22]. The nucleosome appears to be the main obstacle for essential

nuclear processes as it impedes the access of protein factors to the DNA template. The major strategy that the cell uses to overcome the nucleosome barrier is the proper removal of the histone octamer and subsequent deposition onto DNA. Important factors in this multi step phenomenon are the histone chaperones that can assemble nucleosome arrays in vitro in the absence of ATP [23]. Their main function is to serve as histone "donors" during chromatin assembly and as "acceptors" for histones during chromatin disassembly [24]. Some of the histone chaperones exhibit preference for binding to H2A/H2B, such as nucleosome assembly protein 1 (Nap1) and nucleoplasmin, or to H3/H4, namely Asf1, CAF-1, HIRA, Vps75, SET, etc. [25]. Histone chaperones may be specialized to function in chromatin assembly or disassembly during particular processes including transcription elongation or DNA repair [26]. Some chaperones are involved in histone transport or in transfer of histones to other chromatin factors; in yeast Nap1 assists in the nuclear transport of H2A/H2B and facilitates the transfer of the H2A variant-containing dimer Htz1–H2B to the SWR1 remodeling complex for incorporation into chromatin [27–29].

The nonhistone protein HMGB1 is a good candidate for a histone chaperone as it contains in its aminoacid sequence a long 30 aa acidic C terminus which may neutralize the positive charge of the core octamer and prevent histone/DNA aggregation. Our experiments show that in the classical stepwise dialysis method for nucleosome assembly the addition of HMGB1 stimulates more than two times the formation of middle-positioned nucleosomes (Fig. 2). The reconstituted particle did not contain HMGB1 molecule(s) as the its electrophoretic mobility did not differ from the reconstituted nucleosome in the absence of HMGB1 (Fig. 2, compare lanes 1 and 2). For us it was more important to make sure that this property would persist in dialysis free experiment when the reconstitution was possible only in the presence of a chaperone. Under the experimental conditions cited above the addition of HMGB1 protein enhanced the reconstitution of nucleosome in a dose dependant manner while no definite particle was formed in the absence of the protein (Fig. 3). Based on the data already published in the literature we hypothesized that the main target for the stimulating effect of HMGB1 should be the positively charged histone octamer. To check this hypothesis we tested two possible mechanisms for the chaperone function of HMGB1 protein. In the first case we preincubated HMGB1 protein with the prefolded core histones and than the labeled DNA fragment was added. In the second case the HMGB1 was preincubated with the DNA under the same experimental conditions and than the mixture was supplemented with the prefolded core histones. We were surprised by the fact that the nucleosome reconstitution was detected only in the second case which clearly showed that the target of HMGB1 action as chaperone was the DNA fragment (Fig. 4). One possible explanation for the stimulating effect of HMGB1 is the "architectural" property of the protein to associate with the middle of the DNA fragment and to kink it [17]. The acquired V shaped DNA structure is probably conformationally more favorable to wrap around the prefolded histone octamer.

We tested the role of the post-synthetic acetylation on the chaperone function of HMGB1 protein. The presence of acetyl group at Lys 2 [16] decreases strongly the stimulating effect of the protein in the stepwise salt dialysis experiment. The same tendency persisted in the dialysis free experiment. One explanation might be the fact that due to Lys 2 acetylation of HMGB1, the protein binds predominantly to DNA ends and loses its ability to bend DNA and respectively to induce V-shape [17]. Small amount of nucleosome particles is still detected in the presence of acetylated HMGB1 protein (Fig. 4, lane 5). According to statistics based on AFM experiments of acHMGB1/DNA complexes [17], binding to free DNA ends is registered in 75% of all scored complexes versus 25% of molecules with internally bound acetylated HMGB1.

Our data broaden the conception for proteins that facilitate the nucleosome reconstitution. We demonstrate that although the accepted term is "histone chaperone", the target molecule might be DNA as the chaperone function of HMGB1 is accomplished through HMGB1/DNA interaction. The histone chaperones play key role in chromatin regulation by modulating the proper histone localization. Their behavior should not be discussed apart from the activity of the ATP-dependent remodelers that alter the histone-DNA interactions and mobilize the histone octamers [30]. HMGB1 and 2 were found to assist nucleosome mobilization induced by SWI/SNF. Such assistance was observed also with RSC, another remodeler belonging to the SWI/SNF family [31]. A co-remodeling activity of HMGB1 has been demonstrated with remodelers from the ISWI family, namely, ACF, CHRAC, and ISWI [32]. These data indicate that the boosting effect of HMGB1 and 2 on nucleosome mobilization might be their general property, which is realized in a manner independent of the remodeler used.

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