

Chromatin Remodeling by Brahma Motor Unit on Mono-Nucleosome DNA

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Abstract:

Cellular deoxyribonucleic acid (DNA) exists as chromatin, a carefully regulated complex of DNA and proteins. Nucleosomes are the basic units of chromatin organization, and a suite of remodeling protein complexes constantly reorganizes them to allow or restrict access to genetic information. One such complex, Brahma, is abundant in *Drosophila*, but little is understood of its interactions with DNA and nucleosomes. Through gel assays and observation under atomic force microscopy (AFM), we have investigated and characterized binding functionality of the Brahma core motor protein (BRM) with mono-nucleosomes and naked DNA.

Introduction:

Access to genetic information and its expression is restricted in the form of chromatin and the action of regulatory complexes. Several large chromatin remodeling complexes are highly conserved among eukaryotes, including the Brahma complexes belonging to the *trithorax* group (trxG), which are abundant at sites of actively transcribed DNA in *Drosophila* [1]. The remodeling and recruitment mechanisms of Brahma are still poorly understood; although, the complex has been found to be an important activator to trxG proteins [2] and to be closely associated with the nucleosome-assembly protein ASF1 [3]. In *Drosophila*, Brahma complexes contain at least ten subunits, including BRM, a large ATPase motor-unit of the SWI/SNF family. We combined purified BRM with short strands of mono-nucleosome chromatin to investigate its activity directly associated with DNA and the potential for BRM participation in nucleosome remodeling.

Methods:

Yeast DNA of 344 bp (3D), 394 bp (9D), 529 bp (5D), and 606 bp (6D) was used as specified. Nucleosomes were formed by mixing DNA and histone octamers (H8) in a salt dialysis (2 M to 250 mM KCl) at 4°C in 20 mM Tris-HCl, pH 7.5; 1 mM EDTA, pH 8.0; 10 mM DTT, 0.5 mM benzamidine; and subsequently exchanged to a storage buffer (20 mM Tris-HCl, pH 7.5; 1 mM EDTA, pH 8.0; 1 mM DTT). DNA and H8 were combined in molar ratios of 1:1 for 3D, 9D, 5D, and 6D. The presence of mono-nucleosomes was confirmed in 6%PAGE/0.8%agarose gels. BRM (185kDa) was isolated from *Drosophila* embryo

extract and purified by column chromatography. Purified BRM was stored at -80°C in 0.1 M KCL, 0.01% NP40, 0.2 mg/ml Flag peptide + protease inhibitors, and 0.5 mM DTT. DNA and BRM were combined in 50:1 and 4:1 ratios [2] at room temperature for 60 minutes in a remodeling buffer (10 mM Tris-HCl, pH 7.5; 50 mM KCl, 4 mM MgCl₂, 2 mM ATP). BRM was added to the DNA constructs with mono-nucleosomes, to naked DNA, and to naked DNA with 1:1 H8 in solution.

Results:

A bright band of material did not migrate through the gels in samples for which BRM was added to DNA with mono-nucleosomes (Figure 1); this effect was also observed for lanes with BRM and naked DNA in 6% PAGE. The intensity of the band increased with the concentration of BRM (50:1 vs 4:1), and ratios of 4:1 BRM always produced a highly visible band in the loading well. Ratios of 50:1 occasionally yielded no clear difference between the 50:1 lane and the BRM-absent control. Progressively higher concentrations of BRM also increased the brightness of the nucleosome band versus the BRM-absent lane and lanes with lower concentrations. Conversely, the intensity of the naked DNA band was observed to decrease in higher BRM concentrations due to binding of BRM to naked DNA (Figures 2 and 3). In samples for which BRM, naked DNA, and H8 were combined, the sample behaved as though it did not contain H8, and no nucleosome band appeared in the gel.

Conclusions:

We observed BRM to interact with DNA containing nucleosomes and naked DNA in our remodeling conditions. It appears likely from gel results that BRM binds readily to both DNA configurations, and this behavior was observed under AFM with proteins bound to naked DNA and DNA which had at least one nucleosome. Though there have been suggestions that Brahma complexes may assist in nucleosome assembly activity [3], BRM did not facilitate the formation of nucleosomes in mixtures of DNA and histone octamers. The intensity of mono-nucleosomes was lower, however, in samples without BRM than in samples which had the protein. By binding to DNA, BRM may exert a stabilizing effect on nucleosomes, which we observed to disintegrate after long periods at temperatures over 20°C.

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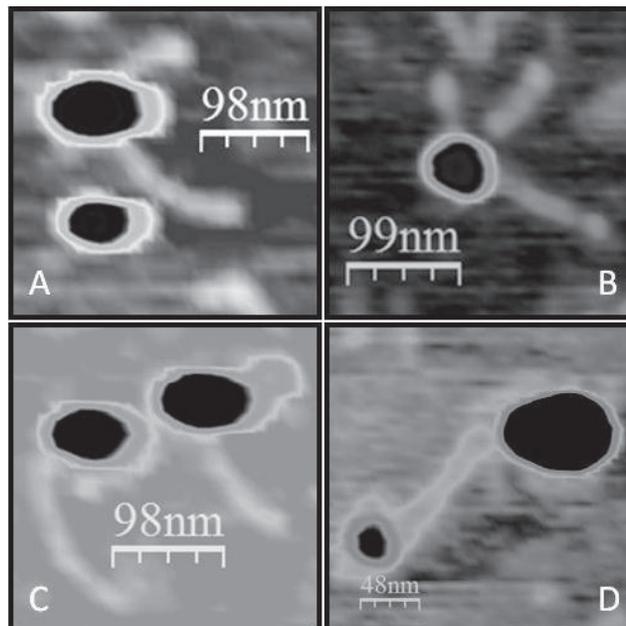


Figure 2: AFM images of BRM and 606 bp DNA (6D). A: Free BRM (lower) and BRM bound to naked DNA (upper). B: BRM bound to naked DNA. C: BRM bound to naked DNA (left) and to DNA with a nucleosome (right). D: BRM on DNA with a nucleosome.

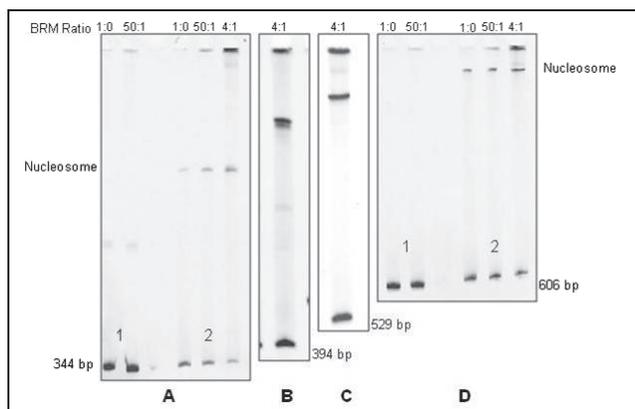


Figure 1: 6% PAGE of BRM with naked DNA and mono-nucleosome samples. A₁, D₁: Ratios of 50:1 DNA:BRM are the lower limit of observable effects on naked DNA. A₂, D₂: Large BRM concentrations (4:1) in nucleosome samples showed a striking increase in the loading well mass and reduction of free DNA, and a moderate increase of nucleosome intensity for 3D and 6D compared to 50:1 and 1:0. B, C: 9D & 5D, respectively, (4:1).

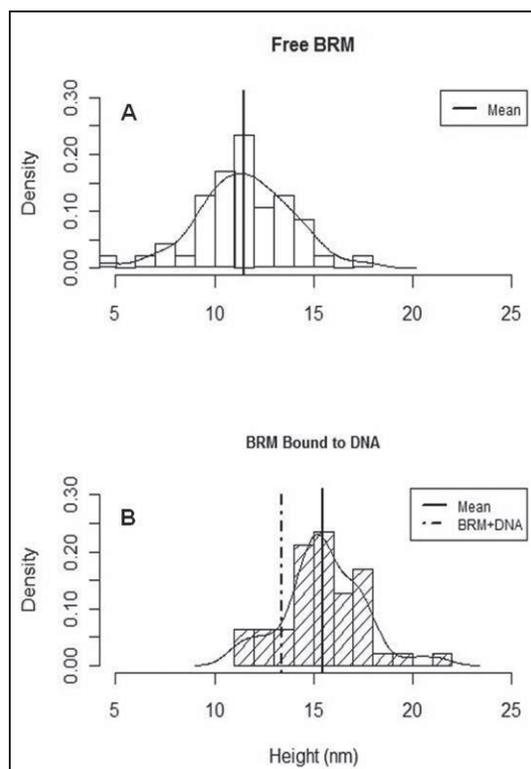


Figure 3: BRM height (nm) distributions measured by AFM. A: Unbound BRM with distribution curve and mean height (11.48 ± 0.703). B: BRM attached to DNA with distribution curve, mean height (15.49 ± 0.585), and predicted height for DNA (1.92 ± 0.0925) added to unbound BRM without conformational effects from binding. Conformational changes from binding were observed ($t_0 = 0$, $t = -4.49$, $P < 0.001$).