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Presence of negative supercoiling in aggregates of histone H1-plasmidic polynucleosome complexes

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Abstract: It was shown in the past that in the presence of histone H1, plasmidic polynucleosomes formed densely packed aggregates. Our current studies demonstrate that these aggregates are susceptible to the actions of E. coli topoisomerase I, human topoisomerase I and DNA nicking enzyme, which is the indication that negative supercoiling is present in the condensed DNA-protein complexes. Since negative supercoiling leads to formation of highly curved and compact plectonemic and toroidal DNA structures, it would be reasonable to assume that DNA negative supercoils are responsible for aggregation of histone H1-plasmidic polynucleosome complexes.

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Histone octamers are structural assemblies of two copies of H2A, H2B, H3, and H4 histones that resemble cylinders in their overall shapes.¹⁻⁴ Within the context of nucleosome core particles, these cylindrical complexes are wrapped around by 146 base-paired DNA segments for 1.67 turns that are upheld mainly by electrostatic interactions and hydrogen bonding.^{4, 5} When plasmid DNA and histone octamers coexisted, circular polynucleosomes will arise, in which each individual nucleosome core particles are separated by linker DNA.⁶⁻⁸ Further studies revealed that these plasmidic polynucleosomes displayed coil-like structures collated in a loose manner.⁶⁻⁸ Upon addition of histone H1, however, these loose assemblies of circular polynucleosomes were transformed instantly into highly compact aggregates (Fig. 1a) as observed from their atomic force microscopic (AFM) images.^{7, 8} In order to acquire information about the transformation from their loose structural entities to densely packed organizations (Fig. 1a), we examined the aggregates of histone H1-plasmidic polynucleosome complexes using Proteinase K, anti-histone H1 antibody and DNA-modification enzymes as probes (Fig. 1b) in our lab recently. Here we report that histone H1-plasmidic polynucleosome aggregates can be slacken by the activities of (1) proteinase K, (2) anti-histone H1 antibody, (3) nicking endonuclease, and (4) human topoisomerase I (human topo I). Most significantly, it is shown in our studies that the action of *E. coli* topoisomerase I (*E. coli* topo I) was capable of causing relaxation of histone H1-plasmidic polynucleosome aggregates, which is evident that negative supercoiling is present in the densely packed DNA-histone complexes.

Figure 1. Schematic illustration of (a) formation of histone H1-plasmidic polynucleosome aggregates from plasmidic polynucleosomes and histone H1 as reported by other groups^{7, 8} and illustrated in the current studies (Fig. S1), and (b) our design for studying histone H1-plasmidic polynucleosome aggregates using Proteinase K, anti-histone H1 antibody and DNA-modification enzymes as probes.

Figure 1 shows schematic presentations of (i) formation of histone H1-plasmidic polynucleosome aggregates from plasmidic polynucleosomes and histone H1⁶⁻⁸ (Fig. 1a) and (ii) our designed examination of these aggregates using enzymes and antibody as probes (Fig. 1b). The plasmid DNA used in the current studies is pAdEasy-1, which was isolated from *E. coli* BJ5183 bacterial cells (Addgene) through following standard protocols^{9, 10} and contains ~33,000 base pairs in its overall sequence (Fig. S1). Plasmidic polynucleosomes were subsequently prepared in our studies through using pAdEasy-1 and core histones as precursors, which displayed relatively loose overall structures in their AFM images (Step 1 in Fig. S1). Once histone H1 was mixed with these preformed plasmidic polynucleosomes, densely packed aggregates were generated, compactness of which was verifiable using AFM (Step 2 in Fig. S1). Our observations of formation of structural aggregates by plasmidic polynucleosomes and histone

H1 (Step 2 in Fig. S1) are coherent with those reported in literature⁶⁻⁸.

With the purpose of ruling out the possibility that the formed histone H1-plasmidic polynucleosome aggregates were undissolvable precipitates, Proteinase K was used first in our studies to probe the highly compact DNA-histone complexes (Fig. 2). Proteinase K is a broad-spectrum protease and is commonly used to remove proteins from nucleic acids¹¹⁻¹³. In theory, if the histone H1-plasmidic polynucleosome aggregates were indeed undissolvable, most of the core histones would be buried within the insoluble mass, which would prevent Proteinase K from accessing to their inner protein molecules. Incubation of histone H1-plasmidic polynucleosome aggregates with Proteinase K was accordingly carried out during our investigations followed by AFM examinations. As seen in the AFM images in Figure 2b, free plasmid DNA was recovered upon Proteinase K's digestion on the histone H1-plasmidic polynucleosome aggregates, which is the sign that (i) histone proteins in these aggregates are accessible by Proteinase K and (ii) histone H1-plasmidic polynucleosome aggregates are not undissolvable precipitates.

Figure 2. Schematic illustration of our examination of responses of histone H1-plasmidic polynucleosome aggregates to Proteinase K (a), and their structural confirmations using AFM (b). A solution containing 100 ng of histone H1-plasmidic polynucleosomes and 2 units of Proteinase K at pH 8.0 was incubated at 37 °C for 30 minutes. Loading of the resultant samples on mica and procedures for further AFM examination are the same as those described in Supplementary data.

It is known, on the other hand, that anti-histone H1 antibody is capable of selectively binding to histone H1 proteins in a tight fashion.^{14, 15} With the intention of determining whether histone H1-plasmidic polynucleosome aggregates could be disturbed by excluding histone H1 from their structures, anti-histone H1 antibody was incubated with these densely packed DNA-histone complexes. Our further AFM studies unveiled that relatively loosen structures were resulted upon incubation of histone H1 antibody with histone H1-plasmidic polynucleosome aggregates (Fig. 3). These observations indicate that aggregation of histone H1-plasmidic polynucleosome complexes was a reversible process upon depletion of their bound histone H1.

Figure 3. Schematic illustration of our examination of responses of histone H1-plasmidic polynucleosome aggregates to anti-histone H1 antibody (a), and their structural confirmation using AFM (b). A solution containing 100 ng of histone H1-plasmidic polynucleosomes and 50 ng of histone H1⁰ antibody at pH 7.0 was incubated at 37 °C for 30 minutes. Loading of the resultant samples on mica and procedures for further AFM examination are the same as those described in Supplementary data.

The above-mentioned Proteinase K and anti-histone H1 antibody are protein-interacting molecules. In order to assess how histone H1-polynucleosome aggregates responds to DNA-interacting enzymes, a DNA nicking endonuclease, Nt.BspQI, was selected next as a probe in our investigations, which is known to generate single-strand scission in the 5'-GCTCTTCN*-3'/3'-CGAGAAGN-5'-containing duplex DNA. Because there are five sites of 5'-GCTCTTCN*-3'/3'-CGAGAAGN-5' in pAdEasy-1, single-strand cleavage in the plasmid should in theory be generable by Nt.BspQI. This nicking endonuclease was consequently allowed to react with histone H1-plasmidic polynucleosome aggregates in our studies, and their resultant products were further analyzed using AFM. In comparison with the original histone H1-polynucleosome aggregates, Nt.BspQI-nicked products displayed relatively relaxed structures in their AFM images (Fig. 4b). These observations signify that single-strand scission can lead to disentanglement of histone H1-plasmidic polynucleosome aggregates.

One of the possibilities that histone H1-polynucleosome aggregates are vulnerable to the action of nicking endonuclease (Fig. 4b) would be the existence of supercoiling in the densely packed DNA-histone complexes because single-strand scission in a circular DNA could lead to a thorough relaxation of DNA supercoils¹⁶. With the aim of clarifying whether supercoiling indeed plays roles in the formation of histone H1-polynucleosome aggregates, human topo I was used next as a probe in our studies. Unlike nicking endonuclease that generates nick sites on duplex DNA, human topo I causes relaxation of both positive and negative DNA supercoils¹⁷. A reaction mixture of histone H1-plasmidic polynucleosome aggregates and human topo I was hence examined using AFM in our investigations. As seen in Figure 4c, AFM images of the resultant products are slack in their backbones, which demonstrated that the histone H1-plasmidic polynucleosome aggregates were susceptible to human topo I's activity. This susceptibility implies that either positive or negative supercoiling is present in the densely packed DNA-histone aggregates. In order to further determine whether it is positive or negative supercoiling that sustains the structures of histone H1-plasmidic polynucleosome aggregates, *E. coli* topo I was chosen next in our investigations. Unlike human topo I that acts on both positively and negatively supercoiled DNA, *E. coli* topo I relaxes only negative DNA supercoils and leaves positive

DNA supercoils undisturbed⁸. This prokaryotic topoisomerase was in turn incubated with histone H1-plasmidic polynucleosome aggregates, which was further examined using AFM. Slackened structural aggregates were observed during our AFM examinations (Fig. 4d), which indicate that histone H1-plasmidic polynucleosome aggregates are responsive to the action of *E. coli* topo I.

Figure 4. Illustration of our examination of responses of histone H1-plasmidic polynucleosome aggregates to (i) Nt.BspQI, (ii) human topo I and (iii) *E. coli* topo I respectively (a), and their structural confirmations using AFM (b, c and d). A buffer solution containing 100 ng of histone H1-plasmidic polynucleosomes at pH 7.0 was incubated separately with 2 units of Nt.BspQI (Fig. 4b), 5 units of human topo I (Fig. 4c), and 5 units of *E. coli* topo I (Fig. 4d) at pH 7.0 at 37 °C for 30 minutes. Loading of the resultant samples on mica and procedures for further AFM examination are the same as those described in Supplementary data.

In order to examine whether any other categories of proteins could affect the histone H1-plasmidic polynucleosome aggregates as well, bovine serum albumin¹⁸ (BSA) was selected next as a probe in our studies, which serves as a negative control. BSA is a large globular protein and has no known role in DNA relaxation.¹⁸ Histone H1-plasmidic polynucleosome aggregates were accordingly incubated with BSA followed by AFM examination. Our results showed that there no structural changes of histone H1-plasmidic polynucleosome aggregates upon their incubation with the serum albumin proteins (Fig. S2). These observations could be indications that integrity of histone H1-plasmidic polynucleosome aggregates is not disrupted by protein molecules that do not cause relaxation of supercoiled DNA.

In conclusion, since negatively supercoiled DNA is the sole substrate of *E. coli* topo I¹⁷, relaxation of histone H1-

polynucleosome aggregates by this prokaryotic topoisomerase (Fig. 4d) is the indication that negative supercoiling is present in the highly condensed DNA-histone complexes. In addition, the outcomes of our studies through using nicking enzymes (Fig. 4b) and human topo I (Fig. 4c) as probes are consistent with the suggestion that supercoiling in the histone H1-polynucleosome aggregates is released by the actions of these two types of DNA-modification enzymes. Because negative supercoiling is always affiliated with formation of highly curved and compact plectonemic and toroidal structures of DNA¹⁹⁻²¹, it would be reasonable to assume that (i) binding of histone H1 to plasmidic polynucleosomes results in the production of negative supercoils and (ii) the produced negative supercoiling subsequently leads to the aggregation of histone H1-plasmidic polynucleosome complexes.

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Supplementary data

Supplementary data associated with this article can be found in the online version.

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