Bioorganic & Medicinal Chemistry Letters xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Quantitative determination of linking number differences between circular polynucleosomes and histone H1-bound circular polynucleosomes

Hao Zhang, Tianhu Li *

Division of Chemistry and Biological Chemistry, School of Physical and Mathematical Sciences, Nanyang Technological University, 21 Nanyang Link, Singapore 637371, Singapore

ARTICLE INFO

Article history:
Received 8 September 2017
Revised 26 October 2017
Accepted 28 October 2017
Available online xxxx

Keywords:
Nucleosome
Histone octamer
Histone H1
Linking number
Polynucleosome

ABSTRACT

With the aim of discovering contribution of histone H1 to linking number changes of DNA, determination of linking number differences between histone H1-free circular polynucleosomes and histone H1-bound circular polynucleosomes was carried out during our investigations. Our results showed that on average, binding of ~11.5 histone H1 molecules causes one linking number change in circular polynucleosomes in the presence of 1.5 mM spermidine. When concentrations of spermidine decreases or increases, these linking number differences decrease significantly. It is therefore evident that linking number changes caused by histone H1 are spermidine concentration-dependent.

As highly abundant proteins in the eukaryotic cells, histones can be divided into core histones (H2A, H2B, H3 and H4) and linker histones (H1/H5) respectively according to their relative positions in nucleosomes.[1,2] Two copies of each of H2A, H2B, H3 and H4 histones constitute histone octamers that display disc-like overall shape.[3] In the co-existence of histone octamers and plasmid DNA, circular polynucleosomes will form, which are the assemblies of repeating unites of nucleosome core particles and linker DNA.[4–6] It was demonstrated in our previous investigations that negative DNA supercoiling was generated by these circular polynucleosomes upon addition of histone H1, which led to their formation of highly compact structural aggregates.[6] Our further studies showed that negative supercoils in these circular polynucleosomes could arise because binding of histone H1 to nucleosomes forced two arms of DNA to adopt right-handed positive supercoils along the surfaces of nucleosomes (Fig. 1).[7] With the intention of knowing linking number changes of DNA that are caused by binding of histone H1 to nucleosomes, new studies on histone H1-bound circular polynucleosomes were carried out in our lab recently. Here we report that in the presence of 1.5 mM spermidine, binding of one histone H1 protein molecule leads to the increase of linking number *ca.* +0.09 in circular polynucleosomes. In addition, when spermidine concentrations either decreased or increased, contribution of histone H1 to linking number changes decreased. In the absence of spermidine or in the presence of 5 mM spermidine, the effect of histone H1 on linking number changes of circular polynucleosomes dropped significantly. It is therefore evident that linking number differences between circular polynucleosomes and histone H1-bound circular polynucleosomes are spermidine concentration-dependent.

Fig. 2a depicts our preparations of (i) nick-containing circular polynucleosome (Structure 3) and (ii) nick-containing histone H1-bound circular polynucleosome (Structure 4) starting with pBR322 (Structure 1). This 4361 base-pair plasmid DNA contains a single 5′-GCTCTTCN*-3′/3′-CGAGAAGN-5′ site in its circular sequence, which is the recognition site of nicking endonuclease, Nt.BspQI. Nt.BspQI was accordingly incubated with pBR322 in our studies for generating a nick site in its circular entity (Structure 2). As shown in Lane 3 in Fig. 2b, rates of mobility shifts of Structure 2 was slower than that of Structure 1 (Lane 2), which is the sign that nick sites were produced in this plasmid DNA. Core histones (H2A, H2B, H3.1, and H4) were incubated next with Structure 2 and the resultant products did not show any mobility in the gel (Lane 4 in Fig. 2b). This non-migratable nature of the band in Lane 4 in Fig. 2b is evident that extremely high molecular weight DNA-protein complexes (Structure 3) were formed. Histone H1-bound polynucleosomes (Structure 4) was subsequently prepared in our studies by incubating Structure 3 with histone H1 (Step 3 in Fig. 2a). Similar to Structure 3 (Lane 4 in Fig. 2b), Structure 4

* Corresponding author.
E-mail address: thli@ntu.edu.sg (T. Li).

https://doi.org/10.1016/j.bmcl.2017.10.072
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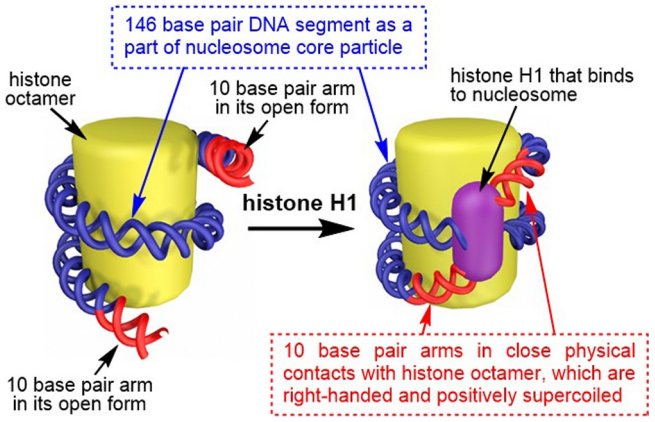

Fig. 1. Illustration of right-handed positive DNA supercoils in the two arm DNA segments that are affiliated with binding of histone H1 to a nucleosome.[7]

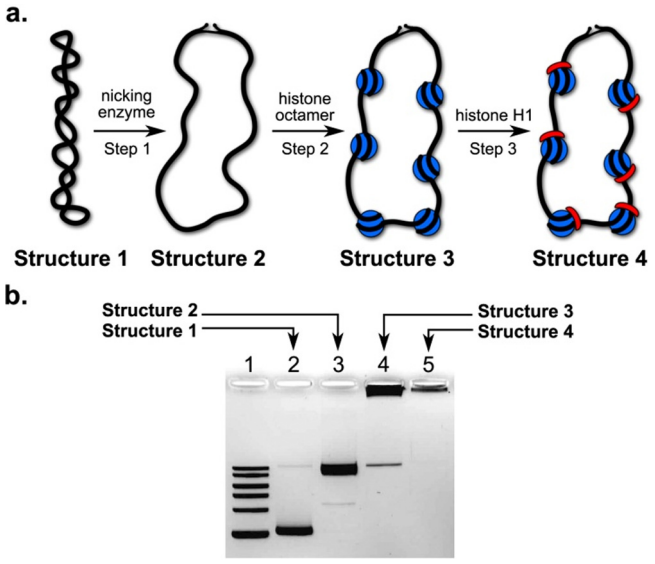

Fig. 2. (a) Illustration of our synthetic routes toward nick-containing histone H1-free polynucleosomes (Structure 3) and nick-containing histone H1-bound polynucleosomes (Structure 4). (b) Agarose gel electrophoretic analysis of Structure 3 and Structure 4. Lane 1, 1 kb DNA molecular weight marker; Lane 2, pBR322 (Structure 1); Lane 3, reaction products (Structure 2) of Nt.BspQI (nicking endonuclease) with pBR322; Lane 4, products (Structure 3) generated from mixtures of Structure 2 and histone octamers; Lane 5, products (Structure 4) generated from mixtures of Structure 3 and histone H1 (detailed descriptions about the sample preparations are given in Supporting Information).

(Fig. 2a) did not display any mobility shift (Lane 5 in Fig. 2b) as a result of formation of pBR322 complexes with histone octamers and histone H1.

With the aim of determining contribution of histone H1 to linking number changes in polynucleosomes, Structure 6 and Structure 8 were prepared in our studies starting with Structure 3 and Structure 4 (Route 1 and Route 2 in Fig. 3a). The nick sites in Structure 3 and Structure 4 were accordingly covalently sealed through using DNA ligase in our studies in the presence of 1.5 mM spermidine (Lane 4 and Lane 5 in Fig. 3b). Proteinase K hydrolysis on the resultant Structure 5 and Structure 6 were carried out next to remove histone proteins from plasmid DNA molecules to form Structure 7 and Structure 8 respectively (Lane 6 and Lane 7 in Fig. 3b). In theory, if histone H1 could contribute to linking number changes of circular DNA in polynucleosomes, linking number difference between Structure 6 and Structure 8 should exist.

If plasmid DNA molecules of identical sequences possess different linking numbers, on the other hand, they are defined as "DNA topological isomers".[8,9] In order to make the distribution patterns of topological isomers of plasmid DNA clearly visible, Shure et al. successfully developed a special agarose gel-based method previously, in which chloroquine molecules were used as DNA intercalating agents. This method reported by Shure et al. was accordingly used in our studies for analyzing distribution patterns of topological isomers in Structure 6 and Structure 8 respectively. As seen in Lane 3 and Lane 4 in Fig. 4a, band distributions emerged in our agarose gel electrophoresis of Structure 6 and Structure 8, as it occurred in all of the previously reported chloroquine-based examination on DNA topological isomers.[10–12] Intensities of these bands in relation to their distances of migration in Lane 3 (Structure 6) and Lane 4 (Structure 8) in Fig. 4a were subsequently measured in our investigation (Fig. 4b and d) through using densitometry methods as reported previously.[13–15] Because relative amounts of each types of DNA topological isomers obtained upon actions of (i) type I topoisomerase,[16,17] (ii) type II topoisomerase,[16,18] and (iii) DNA ligase[14] obey Gaussian distributions,[14,16–18] Gauss fit was performed next in our studies on the data shown in Fig. 4b and d through using the same approaches as those reported by Vetcher et al.[14] for the purpose of generating Gaussian representation of linking number distributions of our DNA topological isomers in Structure 6 (Fig. 4c) and Structure 8 (Fig. 4e) respectively. Our obtained mean values of ΔLk ($\Delta Lk = Lk - Lk_0$, where Lk is the linking number of supercoiled plasmid DNA and Lk_0 is the one in its relax form[8]) in (i) Structure 6 and (ii) Structure 8 derived from the Gaussian distributions in Fig. 4c and e are (i) −5.89 and (ii) −5.38 while the difference between these two mean values is +0.51 [(−5.38) − (−5.89) = +0.51]. The value of +0.51 is the linking number contributed by histone H1 molecules that bound to Structure 8 because supercoils in Structure 8 were contributed by both histone octamers and histone H1 while those in Structure 6 was caused by histone octamers alone. In addition, since Structure 8 (i) possesses 5.89 histone octamers in its structure, and (ii) holds the same quantity of histone octamers as Structure 6, there should be 5.89 histone octamers on average in Structure 8. Consequently, there should be 5.89 histone H1 molecules that bound to nucleosomes in Structure 8. As a result, each histone H1 protein molecule contributes to changes of linking number is +0.09 (+0.51/5.89 = +0.09), which signifies that binding of 11.5 (5.89/0.51 = 11.5) histone H1 molecules produces one linking number change in circular polynucleosomes.

Besides the examinations shown in Fig. 4 that were conducted in the presence of 1.5 mM spermidine, studies of effects of lower concentrations of spermidine was carried out as well during our investigations. As shown in Figs. 1 and 2 in Ref. 19, differences of mean values of linking numbers of DNA topological isomers between histone H1-free polynucleosomes and histone H1-bound polynucleosomes are +0.27 and +0.03 in the presence of 0.75 mM and 0 mM spermidine respectively. These results indicate that differences of linking numbers between (i) histone H1-bound and (ii) histone H1-free polynucleosomes decreased with the decrease of spermidine concentrations. This decrease is consistent with our previous observations that at lower concentrations of spermidine and in the absence of spermidine, nucleosomes tended to adopt their arm-closed forms (Assembly 1 in Column 1 in Fig. 5b).[7] More specifically, since the two arm DNA segments already exist in their arm-closed forms in the absence of spermidine in Assembly 1, binding of histone H1 to nucleosomes (Assembly 2 in Column 1 in Fig. 5b) will have little effects on the conformational changes of nucleosomes.

With the aim of knowing how increase of spermidine concentration affects differences of linking numbers of DNA topological isomers between histone H1-free polynucleosomes and histone