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

Zhang, Hao; Li, Tianhu

2018

Zhang, H., & Li, T. (2018). Quantitative determination of linking number differences between circular polynucleosomes and histone H1-bound circular polynucleosomes. *Bioorganic & Medicinal Chemistry Letters*, 28(3), 537-540. doi:10.1016/j.bmcl.2017.10.072

<https://hdl.handle.net/10356/90151>

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

© 2017 Elsevier Ltd.. All rights reserved. This paper was published in *Bioorganic & Medicinal Chemistry Letters* and is made available with permission of Elsevier Ltd.

Downloaded on 26 Apr 2023 13:35:01 SGT

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

Hao Zhang and 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
Revised
Accepted
Available online

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.

* Corresponding author. Tel.: +65-6513-7364; fax: +65-6791-1961; e-mail: thli@ntu.edu.sg

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.

Figure 1. Illustration of right-handed positive DNA supercoils in the two arm DNA segments that are affiliated with binding of histone H1 to a nucleosome⁷.

Figure 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 Figure 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 Figure 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 (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.

Figure 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).

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.

Figure 3. (a) Illustration of our synthetic routes toward Structure 6 and Structure 8 starting with nick-containing histone H1-free polynucleosomes (Structure 3) and nick-containing histone H1-bound polynucleosomes (Structure 4) separately. (b) Electrophoretic analysis of Structure 6 and Structure 8 as well as their precursors. Lane 1, 1 kb DNA molecular weight marker; Lane 2, Structure 3; Lane 3, Structure 4; Lane 4, reaction products (Structure 5) of DNA ligase with Structure 3; Lane 5, reaction products (Structure 7) of DNA ligase with Structure 4; Lane 6, reaction products (Structure 6) of proteinase K with Structure 5; Lane 7, reaction products (Structure 8) of proteinase K with Structure 7 (detailed descriptions about the sample preparations are given in Supporting Information).

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 Figure 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 isomers¹⁰⁻¹². Intensities of these bands in relation to their distances of migration in Lane 3 (Structure 6) and Lane 4 (Structure 8) in Figure 4a were subsequently measured in our investigation (Fig. 4b and Fig. 4d) through using densitometry methods as reported previously¹³⁻¹⁵. 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 ligase¹⁴ obey Gaussian distributions^{14,16-18}, Gauss fit was performed next in our studies on the data shown in Figures 4b and 4d 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 form⁸) in (i) Structure 6 and (ii) Structure 8 derived from the Gaussian distributions in Figure 4c and Figure 4e 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.

Figure 4. (a) Chloroquine-based agarose gel electrophoretic analysis on Structure 6 and Structure 8. Lane 1, 1 kb DNA molecular weight markers; Lane 2, relaxed forms of pBR322; Lane 3, Structure 6; Lane 4, Structure 8 (detailed descriptions about the sample preparation are given in Supporting Information). (b) Densitometry tracing of gel electrophoretic results in Lane 3 (Structure 6). (c) Plot of Gauss fit on the data shown in Figure 4b, which gave rise to -5.89 as its mean value of ΔLk . (d) Densitometry tracing of gel electrophoretic results in Lane 4 (Structure 8). (e) Plot of Gauss fit on the data shown in Figure 4d, which gave rise to -5.38 as its mean value of ΔLk .

Figure 5. (a) Plot of concentrations of spermidine vs. differences of mean linking numbers between histone H1-free circular polynucleosomes and histone H1-bound circular polynucleosomes (this plot was created based on the data derived from Figure 4 in this article and Figures 1 to 5 in Ref. 19). (b) Illustration of DNA arm conformations of nucleosomes in the presence of 0 mM, 1.5 mM and 5 mM spermidine.

Besides the examinations shown in Figure 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 Figure 1 and Figure 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 H1-bound polynucleosomes, on the other hand, additional studies were carried out during our investigations as well. As shown in Figures 3 to 5 in Ref. 19, in the presence of 2 mM, 3 mM and 5 mM spermidine, linking number differences of DNA topological isomers between histone H1-free polynucleosomes and histone H1-bound polynucleosomes decreased from +0.43 to +0.29 to -0.12 (Fig. 5a). One of the likely causes of this decrease is that at its higher concentrations, spermidine molecules would enforce the DNA segments in nucleosome core particles to widely open up, which will relax left-handed negative supercoils in the DNA segments that enter and exit nucleosome core particles. Once histone H1 binds to nucleosomes, negative supercoils at the ends of nucleosomal DNA and positive supercoils in the two 10 base-pair DNA arms will form at the same time (Column 3 in Fig. 5b). Because the simultaneously formed negative supercoils and positive supercoils cancel out each other upon proteinase K's digestion, difference of linking numbers of DNA topological isomers between histone H1-free polynucleosomes and histone H1-bound polynucleosomes at high spermidine concentrations diminished (Fig. 5a).

If spermidine alone could indeed affect DNA arms of nucleosomes in the manner as portrayed in Figure 5b⁷, their effects on linking numbers of circular polynucleosomes should in theory exist in the absence of histone H1 as well. Correlations of spermidine concentrations with linking numbers of circular polynucleosomes were accordingly examined in our studies

without involvement of histone H1 (Fig. 6). As shown in Figure 6a, Structure 3 was first allowed to form close circular polynucleosomes by the action of DNA ligase in the presence of 0 mM, 0.75 mM, 1.5 mM, 2 mM, 3 mM and 5 mM spermidine respectively, followed by proteinase K hydrolysis. The mean ΔLk values of topological isomers of Structure 30, Structure 32, Structure 34, Structure 36, Structure 38 and Structure 40 were subsequently determined (Fig. 6a in this article and Fig. 6 in Ref. 19) in the same manners as those described in Figure 4. As illustrated in Figure 6b, mean ΔLk values of pBR322 increased when spermidine concentrations increased from 0 mM to 1.5 mM. When spermidine concentrations increased from 2 mM to 5 mM, however, the mean ΔLk values of polynucleosomes decreased (Fig. 6b). These observations are consistent with the suggestion that the arm-open and arm-closed forms of DNA exist in the ways as presented in Figure 5b.

Figure 6. (a) Electrophoretic analysis of circular polynucleosomes prepared in the presence of different concentrations of spermidine. Lane 1, 1kb DNA molecular weight markers; Lane 2, relaxed forms of pBR322; Lane 3 to Lane 8, circular polynucleosomes prepared in the presence of 0 mM (Lane 3), 0.75 mM (Lane 4), 1.5 mM (Lane 5), 2 mM (Lane 6), 3 mM (Lane 7) and 5 mM (Lane 8) spermidine respectively. (b) Plot of spermidine concentrations vs. mean ΔLk values of circular polynucleosomes (data of mean ΔLk values of circular polynucleosomes for Figure 6b are shown in Figure 6 in Ref. 19).

In conclusion, quantitative determination of linking number differences between histone H1-free circular polynucleosomes and histone H1-bound circular polynucleosomes were newly carried out through using chloroquine-based agarose gel electrophoresis. It was demonstrated in our studies that binding of ~11.5 histone H1 proteins to nucleosomes produces a change of one linking number in circular polynucleosomes in the presence of 1.5 mM spermidine. Our studies also showed that in the absence of spermidine or in presence of 5 mM spermidine, difference of linking numbers between histone H1-free and histone H1-bound polynucleosomes falls drastically. These observations indicate that the spatial arrangements of histone H1 in its binding to DNA in circular polynucleosomes can be probed by varying concentrations of spermidine. In addition, since linker DNA and histone H1 are present in entire chromosomes, the handedness of 30 nm fibers, loops, rosettes, coils, chromatids and chromosomes could be determined by DNA arm closure-affiliated negative supercoils that were observed in our studies.

Acknowledgments

This work was supported by Ministry of Education in Singapore and Nanyang Technological University through research grants (MOE2014-T2-2-042, MOE RG14/15 and MOE RG13/16) to Tianhu Li.

Supplementary data

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

References and notes

1. Cutter, A.; Hayes, J. J. *Febs Lett.* **2015**, 589, 2914.

2. Ramakrishnan, V. *Annual review of biophysics and biomolecular structure.* **1997**, 26, 83.
3. Luger, K.; Mader, A. W.; Richmond, R. K.; Sargent, D. F.; Richmond, T. J. *Nature.* **1997**, 389, 251.
4. Hizume, K.; Araki, S.; Yoshikawa, K.; Takeyasu, K. *Nucleic Acids Res.* **2007**, 35, 2787.
5. Rogge, R. A.; Kalashnikova, A. A.; Muthurajan, U. M.; Porter-Goff, M. E.; Luger, K.; Hansen, J. C. *Journal of visualized experiments : JoVE.* **2013**,
6. Zhang, H.; Li, T. *Bioorg Med Chem Lett.* **2017**, 27, 168.
7. Zhang, H.; Li, T. *Bioorg Med Chem Lett.* **2017**, 27, 1149.
8. Bates, A. D.; Maxwell, A. *DNA Topology*: Oxford University Press; 2005.
9. Wang, J. C. DNA Topoisomerases as Targets of Therapeutics: An Overview. *DNA Topoisomerases: Biochemistry and Molecular Biology.* Elsevier Science; 1994.
10. Shure, M.; Pulleyblank, D. E.; Vinograd, J. *Nucleic Acids Res.* **1977**, 4, 1183.
11. Goldstein, E.; Drlica, K. *Proc Natl Acad Sci U S A.* **1984**, 81, 4046.
12. Zechiedrich, E. L.; Khodursky, A. B.; Bachellier, S.; Schneider, R.; Chen, D.; Lilley, D. M.; Cozzarelli, N. R. *The Journal of biological chemistry.* **2000**, 275, 8103.
13. Vologodskii, A. V. *Molekuliarnaia biologiya.* **2001**, 35, 285.
14. Vetcher, A. A.; McEwen, A. E.; Abujarour, R.; Hanke, A.; Levene, S. D. *Biophysical chemistry.* **2010**, 148, 104.
15. Dunlap, D.; Yokoyama, R.; Ling, H.; Sun, H. Y.; McGill, K.; Cugusi, S.; Lucchesi, J. C. *Nucleic Acids Res.* **2012**, 40, 11281.
16. Bates, A. D.; Maxwell, A. *Biochemical Society transactions.* **2010**, 38, 438.
17. Son, L. S.; Bacolla, A.; Wells, R. D. *J Mol Biol.* **2006**, 360, 267.
18. Hardin, A. H.; Sarkar, S. K.; Seol, Y.; Liou, G. F.; Osheroff, N.; Neuman, K. C. *Nucleic Acids Res.* **2011**, 39, 5729.
19. Zhang, H.; Li, T. Dataset on the effects of spermidine on linking number differences between histone H1-free and histone H1-bound circular polynucleosome. Data in brief; (submitted).