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COMMUNICATION

Interference of intrinsic curvature of DNA by DNA-intercalating agents†

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It has been demonstrated in our studies that the intrinsic curvature of DNA can be easily interrupted by low concentrations of chloroquine and ethidium bromide. In addition, the changes of DNA curvature caused by varying the concentration of these two DNA intercalators can be readily verified through using an atomic force microscope.

Unlike canonical-B structures of DNA, intrinsically curved DNA is a type of DNA molecule whose backbone exists in the bent conformation.[1–4] This non-B structure of DNA was discovered in the 1980s and is known to play important roles in various biological processes.[5,6] The DNA sequence in the replication origin in Simian Virus 40, for example, is curved, which is crucial for proteins binding for initiation of replication.[7] In addition, topoisomerase I is known to bind tightly to and selectively cleave some intrinsically curved DNA.[8] These DNA intrinsic curvatures have generally resulted from local structural polymorphism in the region of homopolymeric dA–dT, which is at least 4 base pairs long and the A–T tracts are repeated in phase with the helix screw.[9,10] The extent of these sequence-directed intrinsic curvatures is found to be strongly dependent on temperature and weakly dependent on the ionic strength of the solution.[11] The previous studies on gel mobility shift and differential scanning calorimetry revealed that the curved DNA may pre-melt to uncurved DNA at elevated temperature.[12–14] Although it has been shown that the ionic strength has insignificant effect on DNA curvature, the curvature of the curved DNA is expected to be increased in a higher ionic strength environment by reducing the repulsion between the two DNA strands.[15] Moreover, some of the DNA ligands have been found to affect the intrinsic curvature of DNA.[16–19] Based on our recent analysis of the circularly curved structure of kinetoplast DNA (kDNA) minicircle from trypanosomatid *Crithidia fasciculata*, we speculate that chloroquine and ethidium bromide, as DNA intercalators, could interfere readily with the extent of intrinsic curvature of DNA. Here we report the results of our studies on the interfering effects of DNA intercalating agents on the intrinsic curvature of kDNA, using atomic force microscopy (AFM).[20–31]

Relaxed form of circular kDNA (2515 bp) was accordingly incubated with nicking enzyme, Nb. BtsI, to allow the generation of a nick site on the circular structure of DNA. In order to confirm a nick site was indeed generated in the kDNA, electrophoresis examination was carried out.[32] As shown in Fig. 1(a), the reaction product of kDNA after catalysis by Nb. BtsI (Lane 3) displays a slower mobility shift than that of control kDNA (Lane 2). This observation indicates that a nick site is generated in kDNA by Nb. BtsI.

Both closed circular kDNA and nicked circular kDNA were next examined in our studies using an atomic force microscope. As shown in Fig. 2, the backbones of both closed and nicked circular kDNA molecules in their AFM images are kinked[33] and the backbone self-crossing occurs in around 80% of the DNA molecules. This observation of kinking and self-crossing is presumably associated with the intrinsic curvatures of kDNA.[34–37] In addition, there is no obvious difference between the AFM images of closed (Fig. 2(a)) and nicked (Fig. 2(b)) circular kDNA, which is indicative of the nick site on kDNA having little effect on the overall shape of the circular DNA molecules.

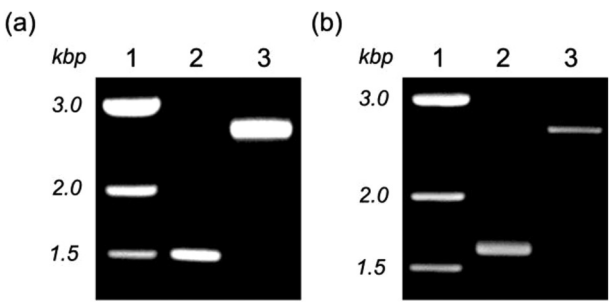

Fig. 1 Electrophoretic analysis of kDNA (a) and pSP73 (b). (a) Lane 1: molecular weight marker; Lane 2: purified closed circular kDNA; Lane 3: kDNA nicked by Nb. BtsI. (b) Lane 1: molecular weight marker; Lane 2: pSP73 alone; Lane 3: pSP73 nicked by Nb. BsrDI. Both electrophoretic analyses were run in 1% agarose gel in 1X tris-acetate–EDTA buffer at 3.3 V cm^{-1} for 3 hours at room temperature with 0.25 μM of ethidium bromide (a), or without ethidium bromide (b).

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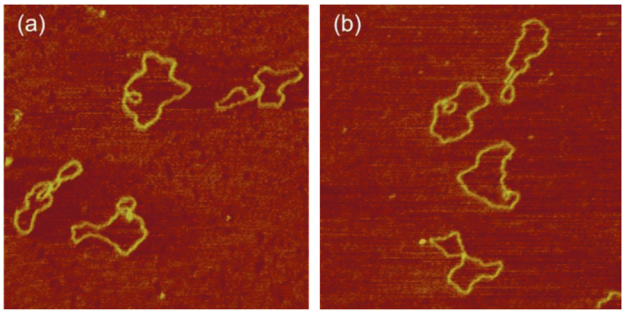

Fig. 2 AFM images (1 μm × 1 μm, *z* range 2 nm) of closed circular kDNA and nicked kDNA. (a) Closed circular kDNA alone. (b) Nicked kDNA alone.

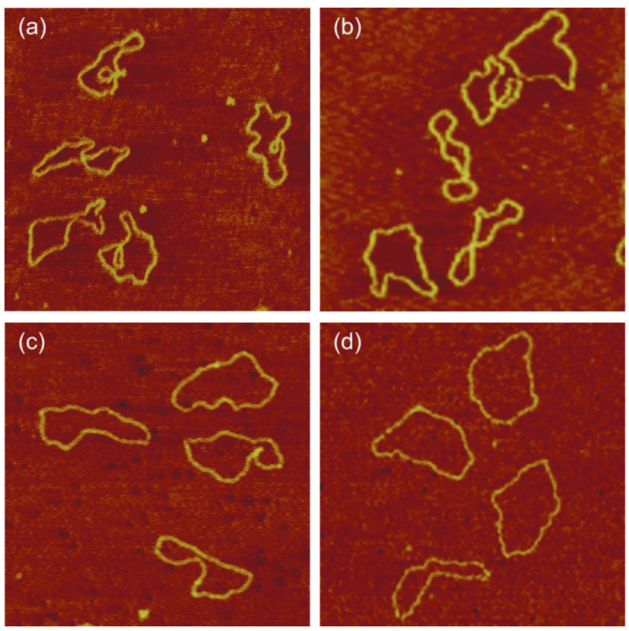

Fig. 3 AFM examination of the effect of chloroquine on the curvature of nicked kDNA. (a) nicked kDNA; (b)–(d) mixtures of nicked kDNA and chloroquine. For (b)–(d), 0.2 nM of nicked kDNA was incubated with 0.4 mM, 0.8 mM and 1.2 mM chloroquine, respectively, for 30 minutes at room temperature before immobilizing sample on mica. The dimension of the images shown are 1 μm × 1 μm, *z* range 3 nm.

Chloroquine is known to be an effective DNA intercalator. With the purpose of examining whether chloroquine could indeed disturb the intrinsic curvature of kDNA, nicked kDNA was incubated with 1.2 mM chloroquine for 30 minutes. The resultant kDNA samples were then examined using AFM. As shown in Fig. 3(d), most of the AFM images of the DNA display smoother and rounder shapes in their backbone as compared with the AFM images of the non-chloroquine intercalating kDNA in Fig. 3(a). The smoother shapes of kDNA shown in Fig. 3(d) could imply that the curvature of DNA was interrupted by the invasion of chloroquine in the molecular structure as anticipated. Since the overall shapes of circular DNA molecules vary upon the addition of DNA intercalators, we wish to introduce "Crossover Distance" and "Minimum Crossover Distance" for describing the curvature alteration in the DNA circles.

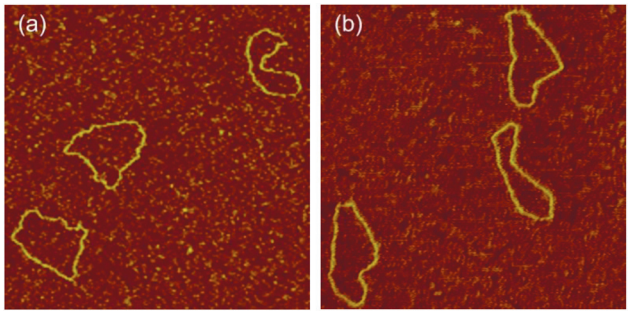

Fig. 4 AFM examination of the effect of chloroquine on nicked pSP73 plasmid. (a) nicked pSP73; (b) mixture of nicked pSP73 with chloroquine. 0.2 nM of nicked pSP73 was incubated with 1.2 mM chloroquine for 30 minutes at room temperature. The dimension of the images shown are 1 μm × 1 μm, *z* range 2 nm.

Crossover Distance for a circular DNA in its AFM image is now defined as the distance between two points on the DNA backbone, on each side of which the number of "on" pixels are approximately equal while Minimum Crossover Distance is the shortest Crossover Distance found in a DNA circle (Fig. S3†). Based on our measurements, the averaged Minimum Crossover Distances for DNA in Fig. 3(a) (kDNA alone) and in Fig. 3(d) (kDNA saturated with chloroquine) are 72.4 nm and 120.2 nm, respectively (Fig. S4†), which indicates that chloroquine could increase the Minimum Crossover Distance of kDNA through its intercalating action. It should be pointed out that the method discussed above is a minimalist approach while quantitative analysis of DNA molecules in their AFM images can in fact be carried out easily and accurately through using "Scanning Probe Image Processor (SPIP)".[38,39]

Furthermore, the chloroquine concentration dependency of the shapes of kDNA was examined during our investigations. As seen in Fig. 3, the smoothness of circular kDNA increased with increasing chloroquine concentration. The observations shown in Fig. 3(b) and 3(c) could be considered as evidence that the interruption of the intrinsic curvature of kDNA is a chloroquine concentration-dependent process. In addition, the averaged Minimum Crossover Distance as a function of concentration of chloroquine was plotted in our studies, which is given in Fig. S4.† Our results revealed that the averaged Minimum Crossover Distance of kDNA increased with the increase in concentration of chloroquine.

pSP73 is plasmid DNA (2464 bp) that contains no identifiable intrinsic curvature in its sequence. To confirm that the kinked shape of kDNA is indeed associated with intrinsic curvature of DNA, nicked pSP73 (Lane 3 in Fig. 1(b)) was examined in our studies. As shown in Fig. 4(a), the nicked pSP73 do not display the kinked shapes and self-crossing structures as observed in nicked kDNA. In addition, when pSP73 were incubated with different concentrations of chloroquine, the shapes of these pSP73 remained the same (Fig. 4(b) and Fig. S1†). These observations are consistent with the suggestion that the kinked structure and self-crossing observed in Fig. 2 are associated with intrinsic curvatures of kDNA.

It is known that ethidium bromide is a more efficient DNA-intercalating agent than chloroquine. With the aim of confirming

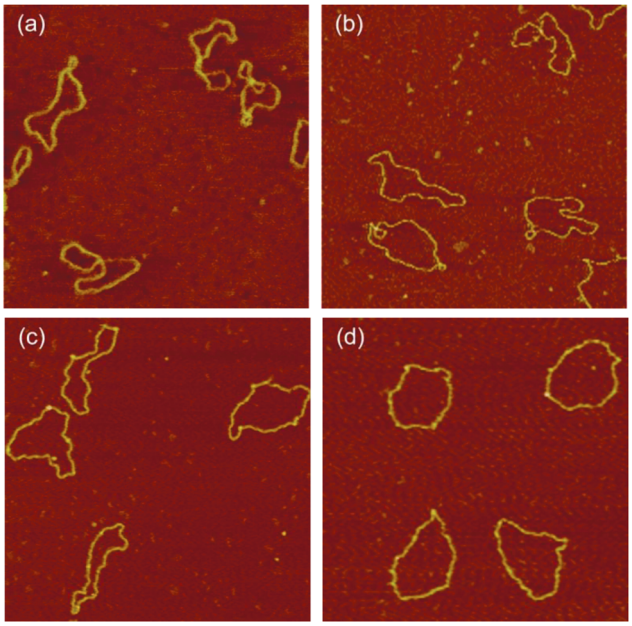

Fig. 5 AFM study of the effect of ethidium bromide on the curvature of nicked kDNA. (a) nicked kDNA; (b)–(d) mixtures of nicked kDNA and ethidium bromide. For (b)–(d), 0.2 nM of nicked kDNA incubated with 1μM, 10 μM and 50 μM ethidium bromide, respectively, for 30 minutes at room temperature before immobilizing sample on mica. The dimension of the images shown are 1 μm × 1 μm, *z* range 2 nm.

that other types of DNA intercalators could disrupt the conformation of DNA curvature as well, incubation of nicked kDNA with ethidium bromide was conducted in our studies. Nicked kDNA was accordingly incubated with ethidium bromide and further examined using AFM. As shown in Fig. 5(a) and 5(d), the backbones of kDNA become more rounded in shape (averaged Minimum Crossover Distance is 135.8 nm) in comparison with the kDNA that was not treated with ethidium bromide (averaged Minimum Crossover Distance is 72.4 nm). This indicates that, similarly to chloroquine, ethidium bromide is capable of interrupting the intrinsic curvature of kDNA as well.

The ethidium bromide concentration dependency experiments carried out in our studies (Fig. 5) showed that the extent of the formation of a smooth circular form of kDNA increased as the concentration of ethidium bromide was gradually increased from 0 μM to 50 μM (see Fig. S5†). The concentration of ethidium bromide used to achieve smooth circular kDNA was 50 μM while the concentration of chloroquine used was 1.2 mM. The lesser concentration of ethidium bromide needed to interrupt the intrinsic curvature of kDNA indicated that ethidium bromide has a higher affinity to intercalate duplex DNA than chloroquine as reported before.[7]

As a control, pSP73 was also examined in our studies (Fig. 6). It is shown in our investigations that there are no obvious changes in the shapes of backbones of pSP73 that was incubated with ethidium bromide (Fig. S2†).

In conclusion, it is demonstrated in our current studies that the intrinsic curvatures of kDNA are able to manifest themselves in their AFM images in the form of kinked and backbone self-crossing-containing structures. On the other hand, in DNA vector

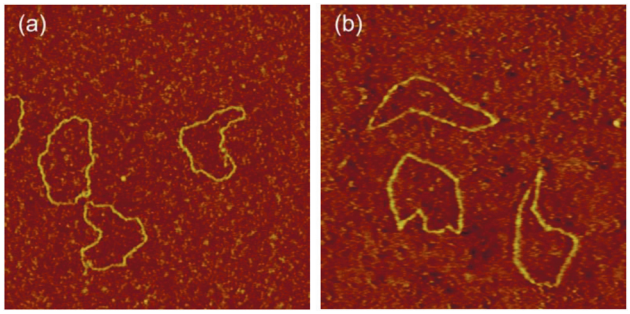

Fig. 6 AFM study of the effect of ethidium bromide on nicked pSP73 plasmid. (a) nicked pSP73; (b) mixture of nicked pSP73 with ethidium bromide. 0.2 nM of nicked pSP73 incubated with 50 μM ethidium bromide for 30 minutes at room temperature. The dimension of the images shown are 1 μm × 1 μm, *z* range 2 nm.

pSP73, which contains no identifiable intrinsic curvature, kinks and self-crossings are unobservable in its duplex backbone under our sample preparation conditions.[40,41] Moreover, our studies show that DNA intercalators such as chloroquine and ethidium bromide can easily interrupt the intrinsic curvature of DNA at their relatively low concentrations. Our above-mentioned observation could have some certain biological implications. Many bacterial promoters, for instance, are intrinsically curved in order to facilitate the binding of RNA polymerase holoenzyme to initiate transcription.[42–44] Consequently, besides its well known role as a mutagen, ethidium bromide should be considered as an agent that could potentially interrupt certain transcription processes *in vivo* through interfering with the intrinsic DNA curvature. Furthermore, since the round shapes of kDNA were achieved by chloroquine and ethidium bromide at different concentrations (1.2 mM for chloroquine and 50 μM for ethidium bromide), the method used in our current studies could in theory be used for estimating the intercalating efficiency of new DNA-intercalating agents.

Acknowledgements

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Notes and references

1 D. M. Crothers, T. E. Haran and J. G. Nadeau, *J. Biol. Chem.*, 1990, **265**, 7093–7096.
2 A. Barbic, D. P. Zimmer and D. M. Crothers, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 2369–2373.
3 Q. Du, M. Vologodskaia, H. Kuhn, M. Frank-Kamenetskii and A. Vologodskii, *Biophys. J.*, 2005, **88**, 4137–4145.
4 J. P. Peters and L. J. Maher, *Q. Rev. Biophys.*, 2010, **43**, 23–63.
5 A. Kanhere and M. Bansal, *Nucleic Acids Res.*, 2003, **31**, 2647–2658.
6 Y. Wada-Kiyama and R. Kiyama, *DNA Res.*, 1996, **3**, 25–30.
7 R. R. Sinden, *DNA Structure and Function*, Academic Press, San Diego, 1994.
8 M. Caserta, A. Amadei, E. Di Mauro and G. Camilloni, *Nucleic Acids Res.*, 1989, **17**, 8463–8474.
9 H. S. Koo, H. M. Wu and D. M. Crothers, *Nature*, 1986, **320**, 501–506.

10 M. Dlakic and R. E. Harrington, *Proc. Natl. Acad. Sci. U. S. A.*, 1996, **93**, 3847–3852.
11 N. C. Stellwagen and Y. Lu, *J. Phys.: Condens. Matter*, 2010, **22**, 494110.
12 P. J. Hagerman, *Biochemistry*, 1985, **24**, 7033–7037.
13 S. S. Chan, K. J. Breslauer, M. E. Hogan, D. J. Kessler, R. H. Austin, J. Ojemann, J. M. Passner and N. C. Wiles, *Biochemistry*, 1990, **29**, 6161–6171.
14 Y. W. Park and K. J. Breslauer, *Proc. Natl. Acad. Sci. U. S. A.*, 1991, **88**, 1551–1555.
15 S. D. Levene, H. M. Wu and D. M. Crothers, *Biochemistry*, 1986, **25**, 3988–3995.
16 F. Barcelo, G. Muzard, R. Mendoza, B. Revet, B. P. Roques and J. B. Lepecq, *Biochemistry*, 1991, **30**, 4863–4873.
17 H. G. Hansma, K. A. Browne, M. Bezanilla and T. C. Bruice, *Biochemistry*, 1994, **33**, 8436–8441.
18 T. Li, Q. Zeng and S. E. Rokita, *Bioconjugate Chem.*, 1994, **5**, 497–500.
19 A. H. F. Lee, A. S. C. Chan and T. Li, *Tetrahedron*, 2003, **59**, 833–839.
20 M. Bezanilla, S. Manne, D. E. Laney, Y. L. Lyubchenko and H. G. Hansma, *Langmuir*, 1995, **11**, 655–659.
21 N. H. Thomson, S. Kasas, B. Smith, H. G. Hansma and P. K. Hansma, *Langmuir*, 1996, **12**, 5905–5908.
22 P. Balagurumoorthy, S. M. Lindsay and R. E. Harrington, *Biophys. Chem.*, 2002, **101–102**, 611–623.
23 L. S. Shlyakhtenko, L. Miloseska, V. N. Potaman, R. R. Sinden and Y. L. Lyubchenko, *Ultramicroscopy*, 2003, **97**, 263–270.
24 J. W. Pavlicek, E. A. Oussatcheva, R. R. Sinden, V. N. Potaman, O. F. Sankey and Y. L. Lyubchenko, *Biochemistry*, 2004, **43**, 10664–10668.
25 Y. Suzuki, Y. Higuchi, K. Hizume, M. Yokokawa, S. H. Yoshimura, K. Yoshikawa and K. Takeyasu, *Ultramicroscopy*, 2010, **110**, 682–688.
26 V. Cassina, D. Seruggia, G. L. Beretta, D. Salerno, D. Brogioli, S. Manzini, F. Zunino and F. Mantegazza, *Eur. Biophys. J. Biophy.*, 2011, **40**, 59–68.
27 D. P. Cavalcanti, D. L. Goncalves, L. T. Costa and W. de Souza, *Micron*, 2011, **42**, 553–559.
28 K. Lund, Y. Liu and H. Yan, *Org. Biomol. Chem.*, 2006, **4**, 3402–3403.
29 R. J. Sha, F. R. Liu, D. P. Millar and N. C. Seeman, *Chem. Biol.*, 2000, **7**, 743–751.
30 H. G. Hansma, K. Kasuya and E. Oroudjev, *Curr. Opin. Struct. Biol.*, 2004, **14**, 380–385.
31 Z. Yang, D. Li and T. Li, *Chem. Commun.*, 2011, **47**, 11930–11932.
32 T. Li, D. Liu, J. Chen, A. H. Lee, J. Qi and A. S. Chan, *J. Am. Chem. Soc.*, 2001, **123**, 12901–12902.
33 W. H. Han, M. Dlakic, Y. W. J. Zhu, S. M. Lindsay and R. E. Harrington, *Proc. Natl. Acad. Sci. U. S. A.*, 1997, **94**, 10565–10570.
34 J. C. Marini, S. D. Levene, D. M. Crothers and P. T. Englund, *Proc. Natl. Acad. Sci. U. S. A.*, 1982, **79**, 7664–7668.
35 J. Griffith, M. Bleyman, C. A. Rauch, P. A. Kitchin and P. T. Englund, *Cell*, 1986, **46**, 717–724.
36 D. W. Li, Z. Q. Yang, G. J. Zhao, Y. Long, B. Lv, C. Li, S. Hiew, M. T. T. Ng, J. J. Guo, H. Tan, H. Zhang and T. H. Li, *Chem. Commun.*, 2011, **47**, 7479–7481.
37 J. C. Marini, P. N. Effron, T. C. Goodman, C. K. Singleton, R. D. Wells, R. M. Wartell and P. T. Englund, *J. Biol. Chem.*, 1984, **259**, 8974–8979.
38 K. J. Neaves, L. P. Cooper, J. H. White, S. M. Carnally, D. T. F. Dryden, J. M. Edwardson and R. M. Henderson, *Nucleic Acids Res.*, 2009, **37**, 2053–2063.
39 R. Subramani, S. Juul, A. Rotaru, F. F. Andersen, K. V. Gothelf, W. Mamdouh, F. Besenbacher, M. D. Dong and B. R. Knudsen, *ACS Nano*, 2010, **4**, 5969–5977.
40 Y. L. Lyubchenko, L. S. Shlyakhtenko and T. Ando, *Methods*, 2011, **54**, 274–283.
41 D. Li, Z. Yang, Y. Long, G. Zhao, B. Lv, S. Hiew, M. T. Ng, J. Guo, H. Tan, H. Zhang, W. Yuan, H. Su and T. Li, *Chem. Commun.*, 2011, **47**, 10695–10697.
42 J. Perez-Martin, F. Rojo and V. de Lorenzo, *Microbiol. Rev.*, 1994, **58**, 268–290.
43 A. K. Cheema, N. R. Choudhury and H. K. Das, *J. Bacteriol.*, 1999, **181**, 5296–5302.
44 F. Gimenes, K. I. Takeda, A. Fiorini, F. S. Gouveia and M. A. Fernandez, *Genet. Mol. Res.*, 2008, **7**, 549–558.

