EPR Study of DNA Mini-Circle Topology

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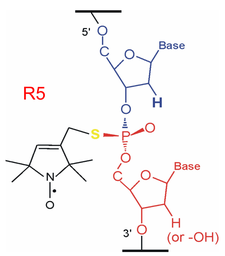
Abstract

DNA can adopt different conformations in solution, and its conformation is high related to its function. DNA minicircles (MCs) are a specific type of circular DNA, and they differ from the corresponding linear constructs by their distinctive topological features, including ring constraint and curvature. MCs provide an ideal system for investigating the DNA shape, which are physical properties of DNA duplexes (e.g., flexibility, bendability) that are collectively determined by sequence and topology (e.g., supercoiling). In addition to those unique properties, MCs of ~ 100 base-pair (bp) has been reported to have a similar radius of curvature as DNA gyres in nucleosomes. Therefore, MCs can be used to isolate the role of DNA shape (curvature in this case) independent from that of the nucleosomal proteins to further study the effects of nucleosome on various protein functions. In this study, we used site-directed spin labeling (SDSL) and continuous-wave (cw) EPR to obtain the motional characterization of a 95-nt single strand minicircle (ssMC) and 95-bp double strand minicircle (dsMC). We successfully developed the method to label MCs with high efficiency (>70%), and the resulting EPR spectra showed that motion in MCs are different from that in linear DNA. This suggests that the DNA topology in MCs is very likely different from linear DNA. This study paved the way for future studies using pulsed EPR distance measurements on 95-bp dsMC, as well as other EPR studies on larger MCs (such as 210-bp MC) and even on DNA plasmid.

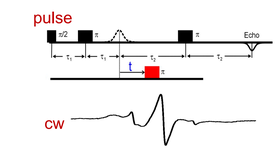
Background

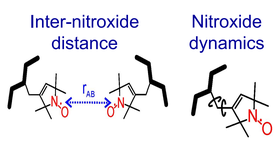
Site-directed spin labeling (SDSL)

1. Attach a nitroxide at a specific DNA site



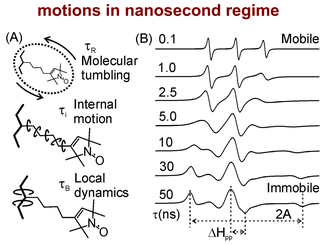
2. Electron Paramagnetic Resonance (EPR) Spectroscopy



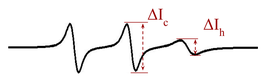


3. Characterize local environment

X-band EPR reports nitroxide motions in nanosecond regime



Quantitatively Characterization of motion



ΔI_c : Center line peak height

ΔI_h : high-field line peak height

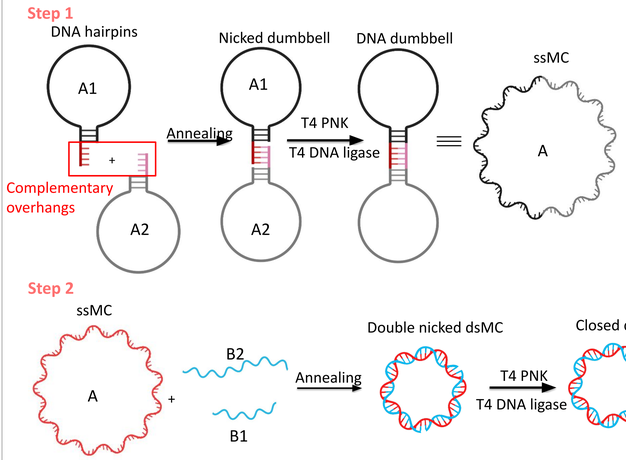
$R = \Delta I_h / \Delta I_c$

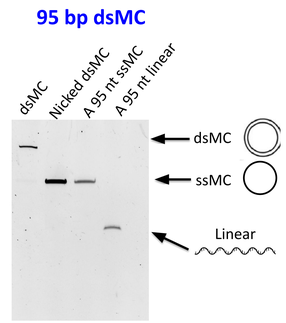
Larger R value => mobile motion

Smaller R value => less mobile motion

Results

(I) Synthesis and Characterization of single strand Mini-circle (ssMC) and double strand Mini-circle (dsMC)

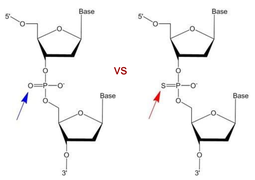




10% Urea PAGE
Fluorescein gel imaging

(II) R5 spin labeling of DNAs

A) DNA modification



A phosphorothiate group was introduced at a specific site on the DNA instead of the normal phosphate group, which serves as the R5 labeling site.

The R5 labeling site in this study is on the A1 strand, PAM-6 site.

PAM PAM-6 site

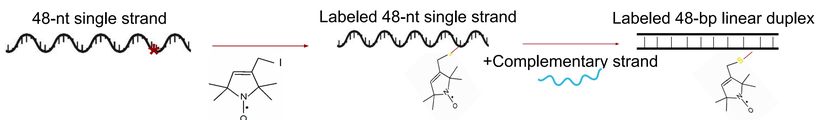
A1 5′-CGATCAAGCCAGTGATAAGTGGAATGCCATGTGGTAA*GATCGGTAGTC-3′

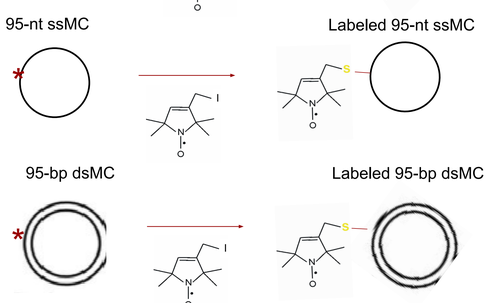
Overhang

*: Phosphorothiate modification

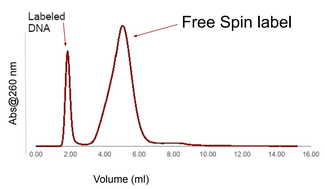
B) Forming ssMC and dsMC using the modified A1 strand

C) R5 labeling of A1 strand, ssMC and dsMC



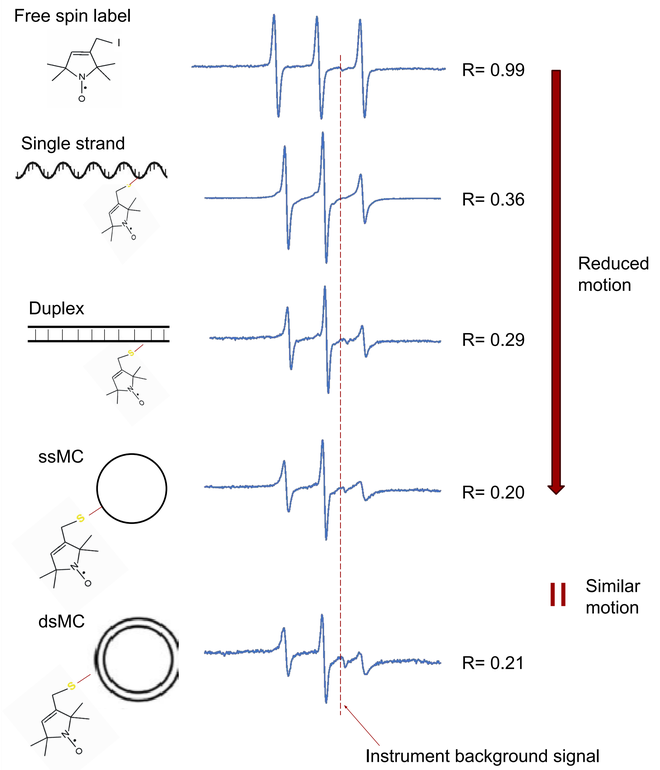


D) Purification of R5 labeled DNA using desalting column and concentrator



Labeling efficiency is >70% for all labeling reactions.

(III) EPR results and analysis



Conclusion

1. Using the R5 spin label, we successfully labeled DNA in different constructs, including single strand DNA, ssMC and dsMC, with high efficiency (>70%).
2. EPR spectra showed that mini-circles have slower motion than linear DNA duplex, and DNA duplex has slower motion than single strand DNA. However, ssMC and dsMC have very similar motion.
3. Since DNA duplex has very similar size as the ssMC, the difference in motion must be originated from local environment difference between linear DNA duplex and circular DNA.

Future Directions

4. Labeling different sites on A1, and/or A2, B1, B2 strands and obtain motion and dynamics information.
5. Labeling **two** sites on 95-bp ssMC and dsMC, and obtain distance information between those sites.
6. Combining the distance information and dynamics information to get a comprehensive understanding of the MC DNA topology and its similarity and difference with linear DNA .
7. Apply the above methodology to other larger MCs (e.g. 210-bp MC). Obtain information on how size of the MC may affect its topology.

CONTACT US

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