Quantitative detection of DNA methylation via the B-Z transition

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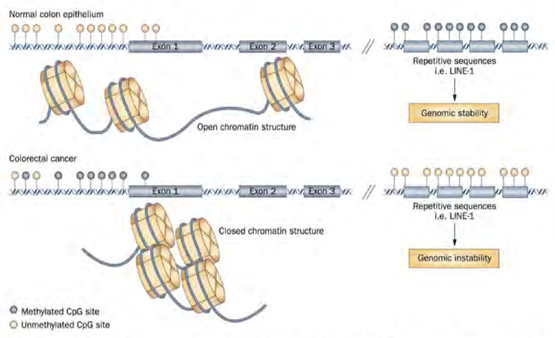
Abstract

DNA methylation is one of the most frequent and critical epigenetic modifications. Abnormal DNA methylation has been implicated in various health issues such as metabolic syndromes and a variety of cancers. It is therefore not only of fundamental interest but also of practical significance to probe the degree of DNA methylation by DNA methyltransferases (DNMT) and to characterize DNA methylation profile. To this end, several methods have been developed.

Here, we propose a new single-molecule-based approach to measure the degree of DNA methylation. It is known that the B-to-Z-DNA transition is sensitive to the extent of DNA methylation: GC repeat sequences with methylated cytosines are easier to convert to Z-DNA than unmethylated, otherwise, identical sequences. Thus, we monitored the B-Z transition occurring to individual DNA molecules in various methylation states by single-molecule FRET and could quantitate DNA methylation in a facile manner and establish the relationship between Z-DNA forming capability and the degree of DNA methylation. Besides, we also tested the effects of various DNMT inhibitors, some of which are clinically tested as anti-cancer drugs, discovering that our technique provides a convenient way to evaluate the efficacy of such drugs.

Introduction

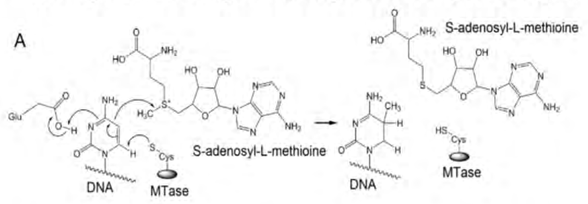
* Epigenetic alterations involving DNA methylation in tumorigenesis. Numerous clinical and experimental data show genome-wide hypomethylation in tumor cells.[1]



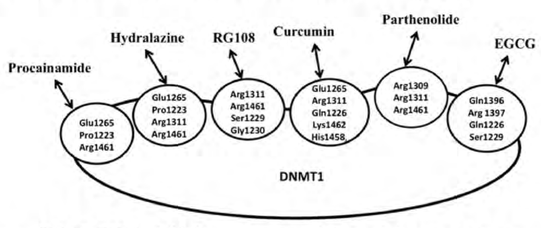
* DNA methyltransferases (DNMTs) in humans and their functions.

DNMTs	Function
DNMT1	Maintaining methylation pattern of DNA after replication. Support DNMT3 in *de novo* methylation. *De novo* methylation of CpG islands in human
DNMT2	Methylation of RNA
DNMT3A	Establishment of *de novo* methylation. Methylated linker DNA between two nucleosomes.

* Methylation of cytosine by DNMT (MTase)[2]



* Inhibition of DNMT1 by various drugs that interact with active site residues of DNMT1 [3,4]

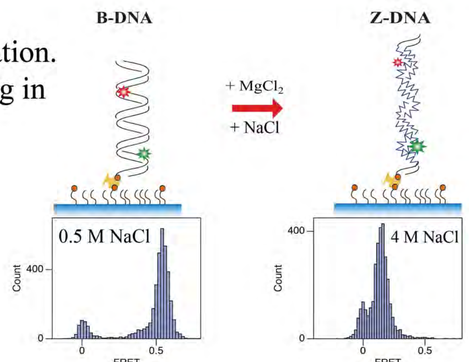


* To monitor DNA methyltransferase activity and screen DNMT inhibitors are of great importance. Several techniques have been developed.
- Radioactive isotope-based assays involve radioactive reagents and tedious processes.
- The bisulfite-based assay requires a longer treatment time and is subject to sample degradation.
- Electrochemical, fluorescent, and colorimetric sensors need use of antibodies and/or complicated experimental procedures and suffer from false-positive results. [5]

Experimental scheme

* Technique

Z-DNA formation depends on the degree of cytosine methylation. Single-molecule FRET can detect the B-Z transition occurring in individual DNA molecules [6,7]

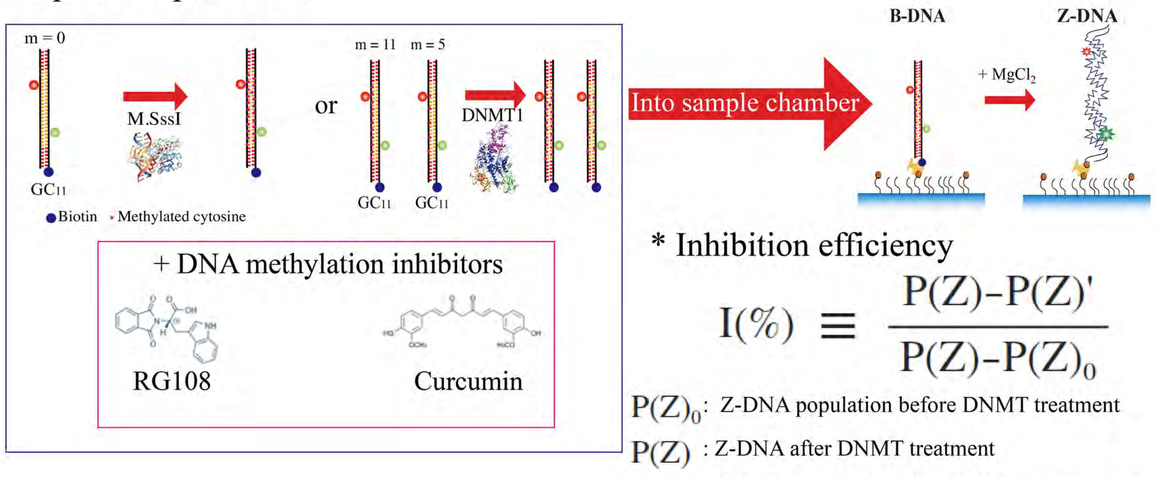


* DNA sequence

$(GC)_{11}$ DNA contains 11 GC repeats with donor(Cy3) and acceptor (Cy5) seperated by 14 bps.
$(GC)_{11}$ in B-form: FRET efficiency(FE) ~ 0.5;
$(GC)_{11}$ in Z-form: FE ~ 0.15.

* Control/start samples with known degrees of DNA methylation
- Non-methylated DNA (m=0); five C methylated in one strand (5); all C methylated in one strand (11); all C methylated in both strands (22)

* DNA methyltransferases for assay: M.SssI (bacterial, NEB) and DNMT1 (human, NEB)
* DNA methylation inhibitors: Curcumin (from curry) and RG108
* Preparation procedure:



* Inhibition efficiency

$$I(\%) \equiv \frac{P(Z)-P(Z)'}{P(Z)-P(Z)_0}$$

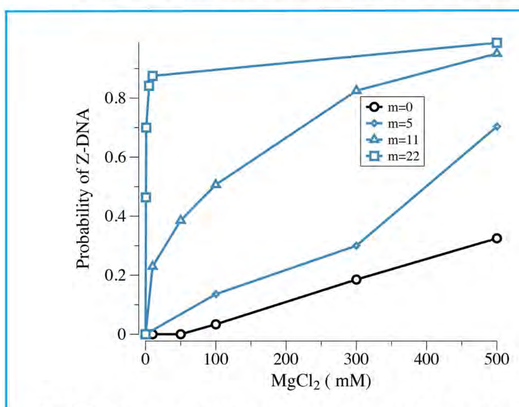
$P(Z)_0$: Z-DNA population before DNMT treatment

$P(Z)$: Z-DNA after DNMT treatment

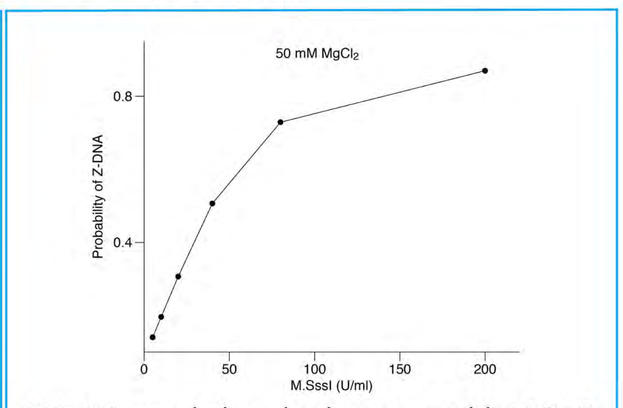
$P(Z)'$: Z-DNA population after DNMT treatment in the presence of inhibitor

Results

* The B-Z transition is sensitive to the degree of DNA methylation.



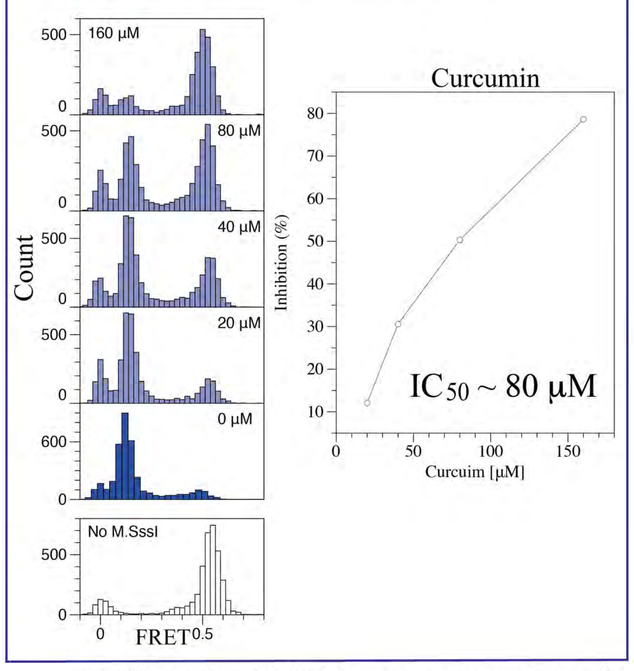
- Z-DNA population increases with salt (Mg^{2+}) concentration and the number(m) of methylated cytosines.



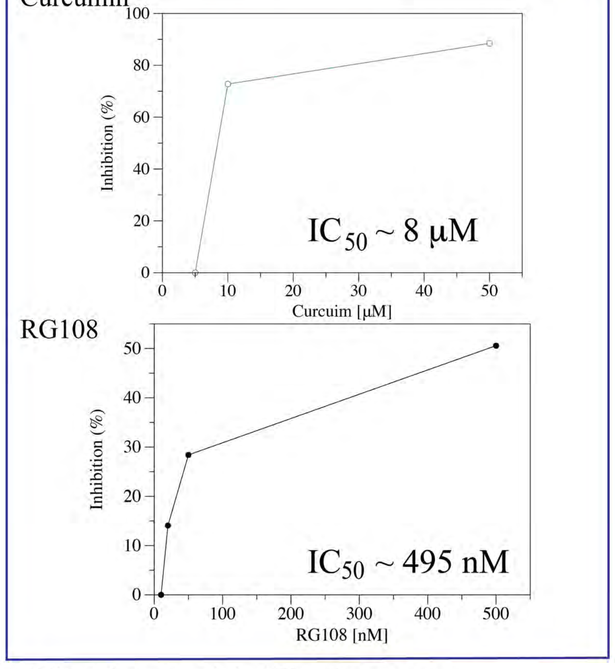
- Z-DNA population also increases with M.SssI concentration in the range of 1 ~ 200 U/ml with the detection limit of 2.5 U/ml.

* Inhibition of DNA methylases (DNMT1 and M.SssI) by different inhibitors.

** Inhibition by curcumin in the presence of 200 U/ml M.SssI & 50 mM $MgCl_2$

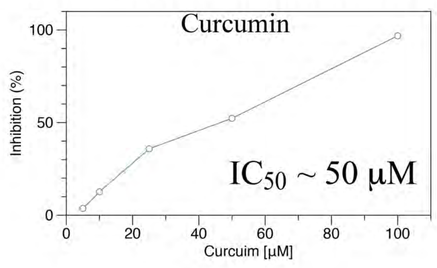


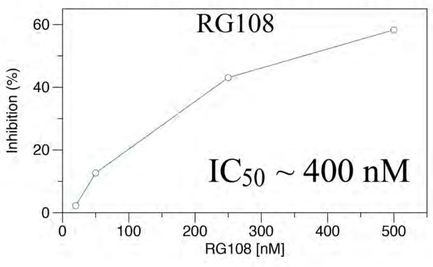
** Inhibition by curcumin &RG108 in the presence of 80 U/ml M.SssI & 50 mM $MgCl_2$



- Half-maximal inhibitory concentration of inhibitor (curcumin), IC_{50}, increases with enzyme concentration (M.SssI).

** Inhibition by curcumin and RG108 in the presence of 100 U/ml DNMT1 & 50 mM $MgCl_2$





- We found that DNMT1 inhibitors also work well with M.SssI and vice versa. Thus, as a primary test, one can use M.SssI to screen inhibitors for DNMT1.

* Advantages of the B-Z transition-based method
- No enzyme work besides DNA methylation assay
- No requirement for SPR or other sophisticated instruments except ordinary TIRF microscope
- Relatively simpler sample design and easier/shorter experimental procedure
- Readily expandable to high-throughput and large-scale assays

Summary

* We suggest a new easy method for quantification of DNMT activity and screening of inhibitors.
* The B-Z transition provides an ideal way to detect and monitor the degree of DNA methylation.
* We confirmed inhibition of DNMTs by RG108 and curcumin.
* Single-molecule FRET makes a highly sensitive technique to measure conformational states of DNA.
* Non-B-DNAs are useful molecular tools to probe molecular, chemical, and environmental conditions.

Reference

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Acknowledgement

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