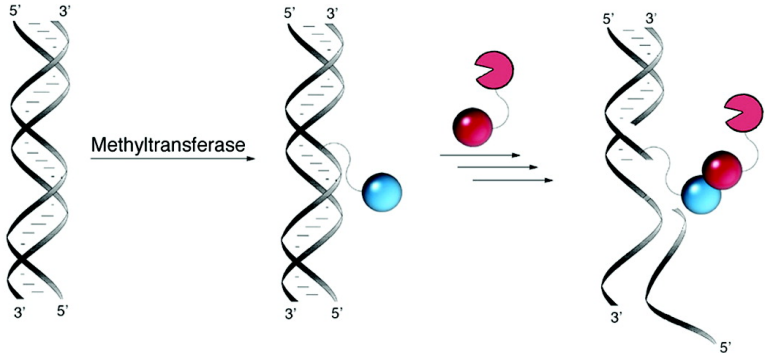
Communication

Methyltransferase-Directed DNA Strand Scission

Lindsay R. Comstock, and Scott R. Rajski

J. Am. Chem. Soc., **2005**, 127 (41), 14136-14137• DOI: 10.1021/ja054128y • Publication Date (Web): 22 September 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 1 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 09/22/2005

Methyltransferase-Directed DNA Strand Scission

Lindsay R. Comstock and Scott R. Rajski*

School of Pharmacy, University of Wisconsin—Madison, 777 Highland Avenue, Madison, Wisconsin 53705-2222

Received June 22, 2005; E-mail: srrajski@pharmacy.wisc.edu

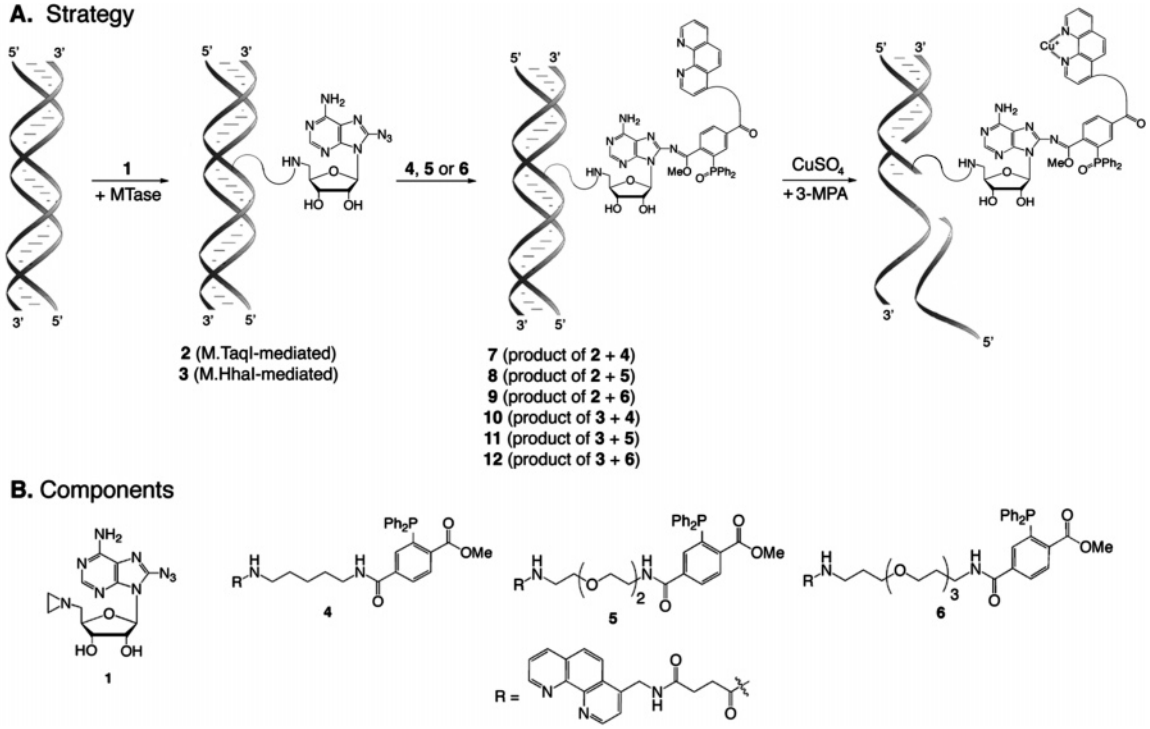
The study of prokaryotic DNA methyltransferases (MTases) has provided significant insight into eukaryotic MTases and the important role that methylation plays in mammalian biology. The prokaryotic enzymes M.TaqI, M.EcoRI, and M.HhaI are all capable of using 5′-aziridine adenylate **1**[1] in place of (*S*)-adenosyl-L-methionine in MTase-dependent DNA alkylation reactions. By virtue of the C8 azide, **1** is significant because it is capable of converting these DNA MTases into azidonucleoside transferases. Not surprising, DNA modified with **1** is capable of undergoing very efficient Staudinger ligation with biotinylated triarylphosphines.[2]

Although one can certainly envision the use of **1** as a means of isolating various MTase substrates from complex mixtures, we expect that **1** might be very useful for quickly identifying regions of DNA hypermethylation. Many methods currently exist by which to do this; however, they are only useful for detection of 5-methylcytosine (5-MeC) and not other modified nucleosides. Aziridine **1** does not have this limitation. How could **1** be used to display regions of DNA methylation? Numerous possibilities exist, including the use of fluorescent tags and various polymerase- and/or exonuclease-based methods. However, we were intrigued by the use of redox-based chemical sequencing methods. In the tradition of Sigman, Tullius, Dervan, and others, we demonstrate here that DNA strand scission, mediated through Haber—Weiss redox cycling, represents an effective means of identifying regions of DNA methylation.[3] The site ordinarily methylated is converted into a DNA strand-cleaving moiety via well-established phenanthroline (OP)—Cu(I) chemistry.[4]

The approach taken is depicted in a generic fashion by Scheme 1. Using a 55 bp oligonucleotide duplex containing the recognition sequences for M.TaqI and M.HhaI, where M.TaqI recognizes the sequence 5′-TCGA-3′ and M.HhaI recognizes the sequence 5′-GCGC-3′ (base normally modified is underlined), production of both **2** and **3** from **1** has been demonstrated.[2,5] Here, it is anticipated that triarylphosphines **4**—**6** will readily undergo the Staudinger ligation with **2** and **3** to afford OP-linked adducts. Importantly, Staudinger ligations involving C8 azido adenosine derivatives produce *O*-methyl imidates depicted by products **7**—**12**.[6] Upon subsequent presentation of **7**—**12** with $CuSO_4$ and the reductant 3-mercaptopropionic acid (MPA), induction of DNA strand scission in a sequence-selective fashion is expected, specifically at the MTase recognition site. Variation of linker lengths in **4**—**6** was originally undertaken because of uncertainties related to copper binding and steric accessibility requirements.

As expected, phosphines **4**—**6** were found to undergo facile Staudinger ligation to both **2** and **3**.[7,8] As shown in Figure 1A, conjugates **7**—**9** produced several scission products upon addition of $CuSO_4$ and MPA. DNA strand scission was induced proximal to the MTase recognition site as determined by comparison to Maxam—Gilbert sequencing lanes. The major products correspond to scission at the A and G residues within the M.TaqI recognition

Scheme 1. Approach and Tools To Achieve MTase-Directed DNA Damage



10.1021/ja054128y CCC: $30.25

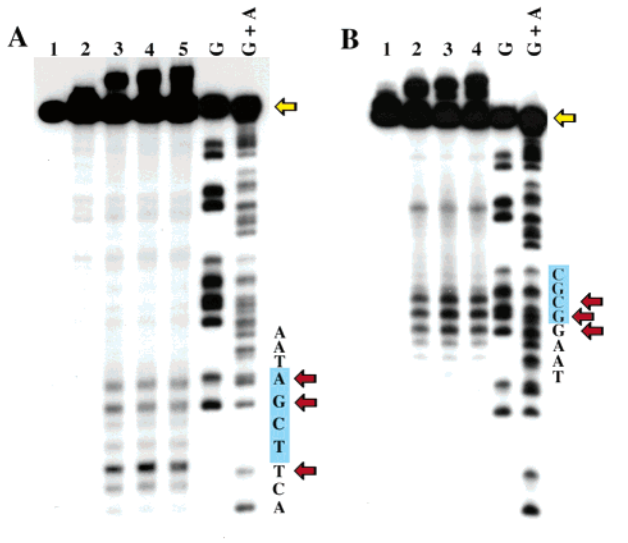


Figure 1. MTase-directed DNA strand scission via Cu(I)-mediated Haber−Weiss chemistry. Strand scission reactions were carried out on **7**−**9** (duplex modified at M.TaqI recognition site) and **10**−**12** (duplex modified at M.HhaI recognition site). Each reaction/lane contained 20 μM $CuSO_4$ and 5.8 mM MPA in 50 mM Tris buffer (pH 8) and was incubated for 2 h at 37 °C. MTase recognition sites are boxed in blue, and major sites of damage are indicated with red arrows. Scission sites were determined by comparison to Maxam−Gilbert sequencing reactions, and native unmodified DNA is indicated with a yellow arrow. (A) DNA modified by M.TaqI: lane 1, DNA standard; lane 2, duplex **2**. Lanes 3, 4, and 5 correspond to conjugates **7**, **8**, and **9**, respectively, following treatment with $CuSO_4$ and MPA. (B) DNA modified by M.HhaI: lane 1, duplex **3**. Lanes 2, 3, and 4 correspond to conjugates **10**, **11**, and **12**, respectively, following treatment with $CuSO_4$ and MPA.

sequence (on the 5′-end-labeled strand), as well as the T residue immediately 5′ of the recognition site.[9] Several other products were observed but are clearly minor contributors. Notably, linker length variation among the OP−phosphine conjugates exerts little, if any, change in product distribution across lanes 3, 4, and 5.

Examination of Figure 1B reveals that adducts **10**−**12**, followed by copper treatment, undergo a distinctive pattern of damage. Strand scission products correlate to the 5′-GC-3′ half of the M.HhaI site, and as with M.TaqI, extensive damage is seen immediately 5′ of the enzyme recognition sequence.[10] Importantly, a significant reduction in scission products is observed as distance from the modified base increases. Again, varying the linker length between the phosphine and OP does not appear to shift the scission site. Additionally, we evaluated the ability of M.SssI[11] to catalytically transfer **1**, and upon ligation with phosphines **4**−**6**, similar strand scission trends were observed compared to those of M.TaqI and M.HhaI.[7]

*The use of agents **1** and **4**−**6** to identify regions of DNA methylation is clearly viable.* However, DNA damage patterns observed for each MTase convey several interesting features. With DNA strand scission being directly linked to DNA modification by **1**, it was anticipated that $(OP)Cu^+$-dependent strand scission (following ligation and Cu(II) reduction) would produce damage both to the 5′ and 3′ side of the MTase-targeted base. The small nature of **1** (and its ligation products) was not expected to afford significant protection of specific regions from oxidative damage as is often the case when using large, high-affinity reagents to deliver strand-cutting moieties. This appears not to be the case, as a marked 5′ selectivity is routinely observed. Moreover, damage is much more localized to the MTase recognition sequence than was originally anticipated. Notably, copper oxene and copper-coordinated hydroxyl radical intermediates have been previously implicated in $(OP)Cu^+$-mediated DNA damage.[3a] The impact of such intermediates would be expected to be influenced by linker length. It is therefore highly interesting that OP−phosphine conjugates **4**−**6** produce almost identical patterns of DNA damage.

For all enzymes evaluated, significant scission is observed in a fashion consistent with damage at the base ordinarily methylated; other damage sites are invariably to the 5′ side of the initially formed "azide-linked" base. Although several additional strand scission events of unequal intensity are often observed to the 5′ side of the recognition site, little or no cleavage to the 3′ side of MTase recognition sites is observed. This biased pattern of strand scission suggests a preferred orientation of the lesion toward the 5′ end of the ^{32}P end-labeled oligonucleotides. Alternatively, it is possible that DNA base modification with **1** (and subsequent ligation partners) predisposes this base, and those to its 5′ side, toward reaction with the reactive oxygen species.

Although considerable work remains to clarify the mechanistic details of these reactions, it is apparent from these studies that ligatable cofactor mimics, such as **1**, represent potentially powerful new tools by which to understand biological methylation.

Acknowledgment. We thank the University of Wisconsin School of Pharmacy and Graduate School for generous funding, and Dr. Derek Robinson (New England Biolabs) for the kind gifts of M.TaqI and M.SssI. L.R.C. acknowledges the American Foundation for Pharmaceutical Education (AFPE) for a Predoctoral fellowship.

Supporting Information Available: Enlarged version of Figure 1 as well as gels with all appropriate controls displayed, M.SssI scission gels, general experimental procedures for the use of **1** with MTases, subsequent ligations with **4**−**6**, and DNA strand scission reactions, as well as preparation and characterization of phosphines **4**−**6**. The complete sequence of the 55 bp substrate is also provided. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Comstock, L. R.; Rajski, S. R. *J. Org. Chem.* **2004**, *69*, 1425.
(2) Comstock, L. R.; Rajski, S. R. *Nucleic Acids Res.* **2005**, *33*, 1644.
(3) Elegantly reviewed in: (a) Sigman, D. S.; Mazumber, A.; Perrin, D. M. *Chem. Rev.* **1993**, *93*, 2295. (b) Pogozelski, W. K.; Tullius, T. D. *Chem. Rev.* **1998**, *98*, 1089.
(4) (a) Gallagher, J.; Chen, C. B.; Pan, C. Q.; Perrin, D. M.; Cho, Y. M.; Sigman, D. S. *Bioconjugate Chem.* **1996**, *7*, 413. (b) Martelli, A.; Constant, J. F.; Demeunynck, M.; Lhomme, J.; Dumy, P. *Tetrahedron* **2002**, *58*, 4291. (c) Chen, C. B.; Sigman, D. S. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 7147. (d) Que, B. G.; Downey, K. M.; So, A. G. *Biochemistry* **1980**, *19*, 5987.
(5) Average yields of MTase-promoted DNA alkylation with **1** are 14% (M.TaqI) and 30% (M.HhaI) based on phosphorimaging. We propose a theoretical yield of 50% in each case; the altered sterics of methylation sites modified with **1** are likely to render each MTase capable of only one alkylation per duplex as opposed to the usual two (one methylation for each strand).
(6) Restituyo, J. A.; Comstock, L. R.; Petersen, S. G.; Stringfellow, T.; Rajski, S. R. *Org. Lett.* **2003**, *5*, 4357.
(7) See Supporting Information.
(8) Average yields of **7**, **8**, and **9** (Scheme 1) are 61, 52, and 55%, respectively. Average yields of conjugates **10**, **11**, and **12** are 69, 78, and 76%, respectively. These represent the percent conversion of alkylated DNA strands to those bearing the pendant phenanthroline moieties represented in Scheme 1.
(9) Phosphorimaging and data processing with ImageQuant revealed the yields for the three major scission products to be 12% (A), 13% (G), and 15% (T). Values are relative to all scission products observed.
(10) The scission yields for the three major scission products are 15% (C), 16% (G), and 13% (G). These data were obtained in a fashion identical to those related to M.TaqI.
(11) (a) Nur, I.; Szyf, M.; Razin, A.; Glaser, G.; Rottem, S.; Razin, S. *J. Bateriol.* **1985**, *164*, 19. (b) Renbaum, P.; Razin, A. *FEBS Lett.* **1992**, *313*, 243.

JA054128Y