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Reversible modification of DNA by methyltransferase-catalyzed transfer and light-triggered removal of photo-caging groups†

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Methyltransferases are powerful tools for site-specific transfer of non-natural functional groups from synthetic analogs of their cosubstrate *S*-adenosyl-L-methionine (AdoMet). We present a new class of AdoMet analogs containing photo-caging (PC) groups in their side chain, enzymatic transfer of PC groups by a promiscuous DNA MTase as well as light-triggered removal from the target DNA. This strategy provides a new avenue to reversibly modulate the functionality of DNA at MTase target sites.

Methyltransferases (MTases) modify several classes of biomolecules post-synthetically and modulate their biological function. In many cases, methylation is dynamic and reversible, indicating a regulatory function, as well documented for histone methylation in epigenetics.[1,2] For DNA, the importance of reversible methylation (m^5C) for controlling transcriptional activity has recently emerged.[3,4] In mRNA, the most abundant internal modification was found to be a reversible methylation (m^6A) with functional implications in mRNA metabolism and translation.[5]

The cosubstrate *S*-adenosyl-L-methionine (AdoMet) serves as nature's methyl donor and synthetic analogs with extended allylic and propargylic groups have proven useful for transferring numerous functional side-chains to specific sites of MTase substrates. The powerful combination of promiscuous or engineered MTases and AdoMet analogs led to a new method for site-specific derivatization of DNA,[6,7] RNA,[8–13] proteins,[7,14,15] and small molecules.[16] Another class of AdoMet analogs, in which the amino acid side chain is replaced by an aziridine, enabled the transfer of the entire cosubstrate.[17] By extending the adenine at the 8 position, transfer of biotin or fluorescent labels directly to DNA was achieved.[17,18] This approach was applied to optically map individual, long DNA molecules and DNA-binding proteins[18] as well as to identify bacteriophage strain types.[19] In the field of RNA, AdoMet analogs were used for fluorescent labeling of tRNA and mRNA but also the transfer of photo-crosslinkers and affinity labels was reported.[8,9,13,20–22]

However, while methylation in nature is often a regulatory process that can be reverted, reversibility of the enzymatically introduced non-natural groups has not been reported, impeding our abilities to study the important dynamic aspects of enzymatic modifications and the downstream processes. On the other hand, light is orthogonal to most biological processes and can be used as trigger to remove photo-caging (PC) groups.[23] Oligonucleotides with PC groups have opened the field of optochemical biology, providing a new level of spatio-temporal control in processes at all levels of gene expression, as exemplified by photo-caged siRNAs, plasmids, triplex forming oligonucleotides and aptamers.[23,24] To date, PC groups are usually installed during chemical synthesis of oligonucleotides and require a combination with ligation strategies to access large nucleic acid molecules such as plasmids in order to bring them under control of light.[25]

Inspired by the enormous potential of optochemical biology, we thought that MTase-catalyzed introduction of PC groups might provide a straightforward route to modify biomacromolecules post-synthetically and reversibly. We therefore designed and synthesized AdoMet analogs for the enzymatic transfer of typical PC groups. Specifically, we appended the *o*-(ONB) and *p*-nitrobenzyl-(PNB), the 4-acetyl-2-nitrobenzyl-(ANB), and the 2-anthraquinonyl-residues (ANT) to *S*-adenosyl-L-homocysteine, yielding AdoMet analogs **1a–d**, termed AdoONB, AdoPNB, AdoANB and AdoANT, respectively (Fig. 1A). For synthesis of the four AdoPCs, *S*-adenosyl-L-homocysteine was reacted with the corresponding bromides, which were synthesized as described previously (Scheme S1, Fig. S1 and S2, ESI†).[26,27] All of those AdoMet analogs contain aromatic ring systems as side-chains, which are bulky and might be incompatible with several MTases. However, our recent work on RNA- and DNA-MTases demonstrated that (i) many highly promiscuous MTases are suitable for this purpose and (ii) the benzyl group promotes enzymatic transfer by promiscuous MTases.[28] In fact, the PNB group was more efficiently transferred from AdoPNB than the

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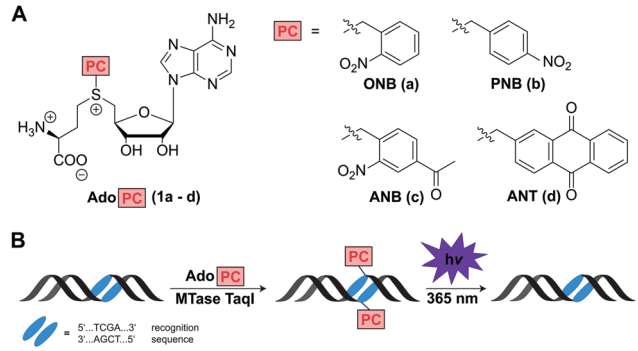

Fig. 1 (A) AdoMet analogs designed for enzymatic transfer and light-triggered removal of functional groups to MTase targets. PC: photo-caging group. (B) Concept of MTase-catalyzed transfer of a photo-cleavable moiety from AdoPC to dsDNA and subsequent photo-cleavage. 2′-Deoxyadenosine of the indicated recognition sequence is modified at the N^6 position by M. TaqI.

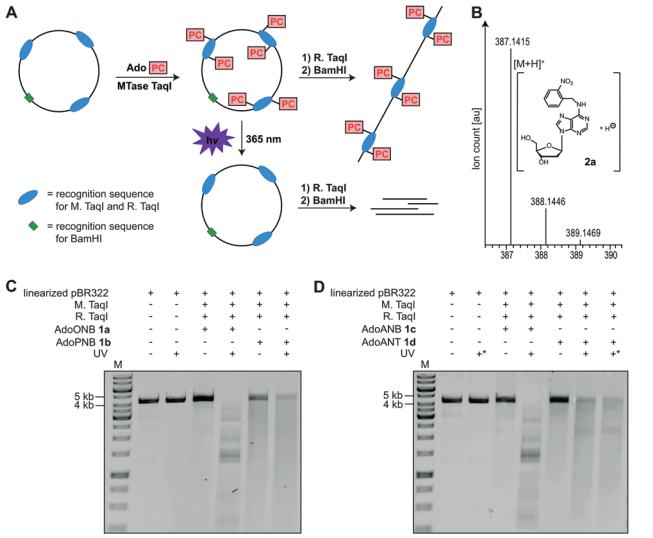

Fig. 2 Enzymatic modification of DNA plasmid pBR322 using MTase TaqI and subsequent photo-cleavage. (A) Concept for the transfer of AdoPC to the N^6 position of 2′-deoxyadenosine using MTase TaqI followed by irradiation with UV light (λ_{max} = 365 nm). For clarity, only three of the seven M. TaqI recognition sites in pBR322 are shown. (B) Mass spectrometric analysis of N^6-ONB modified 2′-deoxyadenosine **2a**. A DNA oligonucleotide was subjected to enzymatic PC-modification, followed by digestion using nuclease P1 and dephosphorylation using FastAP. Calculated mass of $[C_{17}H_{19}N_6O_5]^+$ = 387.1411 [M + H]$^+$, found 387.1415. (C and D) Analysis of enzymatic modification and photo-cleavage of plasmid DNA according to A. Conditions: irradiation at 365 nm for 10 min or 30 min (marked with *), 1% agarose gel (100 V, 1 h), staining with ethidium bromide. M: GeneRuler™ 1 kb DNA ladder (Thermo Fisher Scientific).

methyl group from AdoMet by the guanine *N*7 methyltransferase from the microsporidian parasite *Encephalitozoon cuniculi* (relative specificity of >300%).[28]

Based on this work and literature on DNA MTases[6,7,18,29] we anticipated that **1a–d** would be efficiently converted by the highly promiscuous DNA MTase TaqI, thus protecting the recognition site from enzymatic cleavage by the corresponding restriction enzyme R. TaqI. Irradiation by light (365 nm) should remove the ONB-,[30,31] ANB-,[27] and ANT-group[32,33] resulting in the first reversible, non-natural modification of DNA that can be enzymatically installed (Fig. 1B).

To test whether **1a–d** are accepted by promiscuous MTases, we used a short dsDNA substrate, M. TaqI and **1a–d**, followed by enzymatic degradation to single nucleosides and LC–MS-based analysis. In all cases, the N^6-modified 2′-deoxyadenosine (**2a–d**) was observed, indicating successful enzymatic installation of the respective PC-group (Fig. 2B and Fig. S7–S9, ESI†).

Next, we used plasmid DNA (pBR322) containing seven M. TaqI recognition sites and carried out enzymatic modification with **1a–d**, followed by enzymatic digest using R. TaqI and plasmid linearization (Fig. 2A). Similar to modification with natural AdoMet, modification using **1a–d** protected pBR322 from degradation by R. TaqI, indicating that all seven recognition sites are efficiently modified, with relative efficiencies AdoMet ≫ AdoANT > AdoPNB > AdoONB > AdoANB (Fig. 2C, D and Fig. S5, S6, ESI†).

To test whether the PC-groups can be removed by light, the enzymatically modified plasmids were irradiated at 365 nm using an LED followed by R. TaqI digestion. We observed that **1a**-treated samples containing the ONB-group at the N^6 of 2′-deoxyadenosine were cleaved by R. TaqI after 10 minutes of UV irradiation (Fig. 2C), suggesting that the ONB-group was removed. A control showing unmodified pBR322 plasmid after UV irradiation demonstrated that degradation is not the result of the UV irradiation itself. The PNB-group however was not photo-cleavable, as **1b**-treated plasmid did not become susceptible to R. TaqI degradation after UV-irradiation (Fig. 2C). The ANB-group could also be removed by light, as plasmid treated with **1c** became susceptible to R. TaqI-based restriction after irradiation at 365 nm for 10 min (Fig. 2D). Results obtained with the ANT-group were ambiguous: irradiation of plasmid treated with **1d** decreased the amount of full-length DNA. However, the restriction products were longer than expected for complete cleavage – even when the irradiation time was extended to 30 min – suggesting that not all of the ANT-groups were removed.

These data indicate that the ONB-group and the ANB-group can be efficiently removed from the N^6 position of 2′-deoxyadenosine by irradiation at 365 nm, in line with previous reports where the 1-(*o*-nitrophenyl)-ethyl (NPE) group was synthetically installed at the N^6 position of 2′-deoxyadenosine.[34,35] The PNB-group appears to be unsuitable as photo-caging group, as irradiation only decreased the amount of full-length DNA, but without leading to defined shorter bands in line with previous reports.[36] The ANT-group has been reported as PC-group for alcohols,[32] aldehydes,[2,37] ketones,[2] carboxylic acids[33] and cAMP.[38] When appended to the N^6 of 2′-deoxyadenosine we observed only partial removal. Nevertheless, the ANT-group might become also useful for this purpose if substitutions facilitating cleavage are found.

In summary, we present the first enzymatic strategy for reversible post-synthetic modification of DNA. Based on our previous observation that the benzyl group is a privileged moiety for MTase-catalyzed transfer, we developed a new class

of AdoMet analogs containing PC-groups in their side-chain and demonstrated transfer to MTase-target sites of DNA. As expected, PC groups block enzymatic cleavage of plasmid DNA by restriction enzymes. Upon irradiation with light, two of these groups – namely the *o*-nitrobenzyl- and the 4-acetyl-2-nitrobenzyl groups – are efficiently removed and the DNA is again accessible for restriction enzymes. By combining enzymatic transfer, synthetic cosubstrates and photo-caging groups, our strategy presents a new avenue for the reversible modulation of biological functions of DNA that can be readily extended to other biomacromolecules such as RNA and proteins.

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Conflicts of interest

There are no conflicts to declare.

Notes and references

1 R. J. Klose and Y. Zhang, *Nat. Rev. Mol. Cell Biol.*, 2007, **8**, 307.
2 J.-Y. Yu, W.-J. Tang, H.-B. Wang and Q.-H. Song, *J. Photochem. Photobiol., A*, 2007, **185**, 101.
3 S. Schiesser, B. Hackner, T. Pfaffeneder, M. Müller, C. Hagemeier, M. Truss and T. Carell, *Angew. Chem., Int. Ed.*, 2012, **51**, 6516.
4 M. Münzel, D. Globisch and T. Carell, *Angew. Chem., Int. Ed.*, 2011, **50**, 6460.
5 B. X. S. Zhao, I. A. Roundtree and C. He, *Nat. Rev. Mol. Cell Biol.*, 2017, **18**, 31.
6 C. Dalhoff, G. Lukinavicius, S. Klimasauskas and E. Weinhold, *Nat. Chem. Biol.*, 2006, **2**, 31.
7 G. Lukinavičius, V. Lapienė, Z. Staševskij, C. Dalhoff, E. Weinhold and S. Klimašauskas, *J. Am. Chem. Soc.*, 2007, **129**, 2758.
8 Y. Motorin, J. Burhenne, R. Teimer, K. Koynov, S. Willnow, E. Weinhold and M. Helm, *Nucleic Acids Res.*, 2011, **39**, 1943.
9 M. Tomkuviene, B. Clouet-d'Orval, I. Cerniauskas, E. Weinhold and S. Klimasauskas, *Nucleic Acids Res.*, 2012, **40**, 6765.
10 J. M. Holstein, D. Stummer and A. Rentmeister, *Protein Eng., Des. Sel.*, 2015, **28**, 179.
11 J. M. Holstein, D. Stummer and A. Rentmeister, *Chem. Sci.*, 2015, **6**, 1362.
12 J. M. Holstein, D. Schulz and A. Rentmeister, *Chem. Commun.*, 2014, **50**, 4478.
13 D. Schulz, J. M. Holstein and A. Rentmeister, *Angew. Chem., Int. Ed.*, 2013, **52**, 7874.
14 K. Islam, W. Zheng, H. Yu, H. Deng and M. Luo, *ACS Chem. Biol.*, 2011, **6**, 679.
15 K. Islam, I. Bothwell, Y. Chen, C. Sengelaub, R. Wang, H. Deng and M. Luo, *J. Am. Chem. Soc.*, 2012, **134**, 5909.
16 S. Singh, J. J. Zhang, T. D. Huber, M. Sunkara, K. Hurley, R. D. Goff, G. J. Wang, W. Zhang, C. M. Liu, J. Rohr, S. G. Van Lanen, A. J. Morris and J. S. Thorson, *Angew. Chem., Int. Ed.*, 2014, **53**, 3965.
17 G. Pljevaljčić, F. Schmidt and E. Weinhold, *ChemBioChem*, 2004, **5**, 265.
18 S. Kim, A. Gottfried, R. R. Lin, T. Dertinger, A. S. Kim, S. Chung, R. A. Colyer, E. Weinhold, S. Weiss and Y. Ebenstein, *Angew. Chem., Int. Ed.*, 2012, **51**, 3578.
19 A. Grunwald, M. Dahan, A. Giesbertz, A. Nilsson, L. K. Nyberg, E. Weinhold, T. Ambjornsson, F. Westerlund and Y. Ebenstein, *Nucleic Acids Res.*, 2015, **43**, e117.
20 F. Muttach, F. Mäsing, A. Studer and A. Rentmeister, *Chem. – Eur. J.*, 2017, **23**, 5988.
21 J. M. Holstein, F. Muttach, S. H. H. Schiefelbein and A. Rentmeister, *Chem. – Eur. J.*, 2017, **23**, 6165.
22 J. M. Holstein, L. Anhäuser and A. Rentmeister, *Angew. Chem., Int. Ed.*, 2016, **55**, 10899.
23 C. Brieke, F. Rohrbach, A. Gottschalk, G. Mayer and A. Heckel, *Angew. Chem., Int. Ed.*, 2012, **51**, 8446.
24 Q. Y. Liu and A. Deiters, *Acc. Chem. Res.*, 2014, **47**, 45.
25 J. Hemphill, J. Govan, R. Uprety, M. Tsang and A. Deiters, *J. Am. Chem. Soc.*, 2014, **136**, 7152.
26 M. Su, J. Wang and X. Tang, *Chem. – Eur. J.*, 2012, **18**, 9628.
27 L. Kammari, T. Solomek, B. P. Ngoy, D. Heger and P. Klan, *J. Am. Chem. Soc.*, 2010, **132**, 11431.
28 F. Muttach, N. Muthmann, D. Reichert, L. Anhäuser and A. Rentmeister, *Chem. Sci.*, 2017, **8**, 7947.
29 C. Dalhoff, G. Lukinavicius, S. Klimasauskas and E. Weinhold, *Nat. Protoc.*, 2006, **1**, 1879.
30 J. A. Barltrop, P. J. Plant and P. Schofield, *Chem. Commun.*, 1966, 822.
31 P. Klan, T. Solomek, C. G. Bochet, A. Blanc, R. Givens, M. Rubina, V. Popik, A. Kostikov and J. Wirz, *Chem. Rev.*, 2013, **113**, 119.
32 T. Furuta, Y. Hirayama and M. Iwamura, *Org. Lett.*, 2001, **3**, 1809.
33 M.-G. Ren, N.-M. Bi, M. Mao and Q.-H. Song, *J. Photochem. Photobiol., A*, 2009, **204**, 13.
34 M. C. Buff, F. Schäfer, B. Wulffen, J. Müller, B. Pötzsch, A. Heckel and G. Mayer, *Nucleic Acids Res.*, 2010, **38**, 2111.
35 F. Schäfer, K. B. Joshi, M. A. Fichte, T. Mack, J. Wachtveitl and A. Heckel, *Org. Lett.*, 2011, **13**, 1450.
36 P. Wan and K. Yates, *Can. J. Chem.*, 1986, **64**, 2076.
37 R. G. Brinson and P. B. Jones, *Org. Lett.*, 2004, **6**, 3767.
38 T. Furuta, H. Torigai, M. Sugimoto and M. Iwamura, *J. Org. Chem.*, 1995, **60**, 3953.