Bioorganic & Medicinal Chemistry Letters 28 (2018) 2189–2194

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Insight into the recognition mechanism of DNA cytosine-5 methyltransferases (DNMTs) by incorporation of acyclic 5-fluorocytosine (^{F}C) nucleosides into DNA

Shohei Utsumi [a], Kousuke Sato [b,*], Satoshi Ichikawa [a,c,*]

[a] Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan
[b] Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Kanazawa 1757, Tobetsu, Ishikari-gun, 061-0293, Japan
[c] Center of Research and Education on Drug Discovery, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan

ARTICLE INFO

Article history:
Received 31 March 2018
Revised 27 April 2018
Accepted 3 May 2018
Available online 5 May 2018

Keywords:
Epigenetic
Acyclic nucleosides
5-fluoro-2′-deoxycytidine
Covalent bond
DNA methyltransferase

ABSTRACT

DNA cytosine-5 methyltransferase (DNMT) catalyzes methylation at the C5 position of cytosine in the CpG sequence in double stranded DNA to give 5-methylCpG (mCpG) in the epigenetic regulation step in human cells. The entire reaction mechanism of DNMT is divided into six steps, which are scanning, recognition, flipping, loop locking, methylation, and releasing. The methylation and releasing mechanism are well-investigated; however, few reports are known about other reaction steps. To obtain insight into the reaction mechanism, we planned the incorporation of acyclic nucleosides, which make it easy to flip out the target nucleobase, into oligodeoxynucleotides (ODNs) and investigated the interaction between the ODN and DNMT. Here, we describe the design and synthesis of ODNs containing new acyclic 5-fluorocytosine nucleosides and their physiological and biological properties, including their interactions with DNMT. We found that the ODNs containing the acyclic 5-fluorocytosine nucleoside showed higher flexibility than those that contain 5-fluoro-2′-deoxycytidine. The observed flexibility of ODNs is expected to influence the scanning and recognition steps due to the decrease in helicity of the B-form.

DNA methylation has a profound effect on gene expression, particularly in gene promoter regions.[1,2] There are a number of repetitive DNA sequences, and some genes are hypermethylated, leading to silencing of the gene.[3–5] Unusual DNA methylation patterns are found in tumor cells, and their inhibition could be a promising target in anticancer drug development. Methylation at the C5 position of cytosine in a CpG sequence in double stranded DNA is catalyzed by DNA cytosine-5 methyltransferase (DNMT) to give 5-methylCpG (mCpG), using *S*-adenosyl-l-methionine (SAM) as a methyl group donor. The whole reaction of DNMT is shown in Fig. 1A.[6,7] Genome DNA is associated with DNMT in a sequence independent manner and scans its recognition site (a: scanning). After finding its specific recognition site, DNMT forms a complex (b: recognition) and then makes a transition to flip out the complex (c: flipping). The resulting complex causes an induced fit to the loop locking conformation for the methylation reaction (d: loop locking). Then, the methylation reaction and releasing of the methylated DNA occur (e and f: methylation and releasing). The methylation and releasing mechanism have been investigated well, as shown in Fig. 1B. First, the Glu residue in the catalytic pocket of DNMT protonates the nitrogen in the cytosine, and a thiol of the Cys residue in the active site acts as a nucleophile and attacks the C6 position of the cytosine to form an enzyme-linked intermediate. Then, the C5 position of the cytosine is methylated by SAM. Upon deprotonation of the C5 position, β-elimination of the added Cys residue occurs to complete the reaction. Contrary to the methylation and releasing steps, little is known about other reaction steps, which are scanning, recognition, flipping and loop locking (Fig. 1A(a)–(d)).[8] Ring-opening of the ribose of the nucleoside and its incorporation into an oligodeoxynucleotide (ODN) increases the flexibility at the modification site and decreases the thermal stability of duplex.[9–11] The flexibility at the acyclic nucleotide site would easily flip out its nucleotide by DNMT (Fig. 1A(c)), and the decrease in thermal stability of the modified ODN would influence the interaction of ODN and DNMT by the conformational change of ODN (Fig. 1A(a) and (b)). To eliminate the discussion of the releasing step (Fig. 1A (f)), which could cause concern with thermal stability of the duplex, 5-fluorocytosine (^{F}C) is used as a nucleobase in our study;

* Corresponding authors at: Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Kanazawa 1757, Tobetsu, Ishikari-gun, 061-0293, Japan (K. Sato) and Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan (S. Ichikawa).
E-mail address: ksato@hoku-iryo-u.ac.jp (K. Sato).

https://doi.org/10.1016/j.bmcl.2018.05.008
0960-894X/

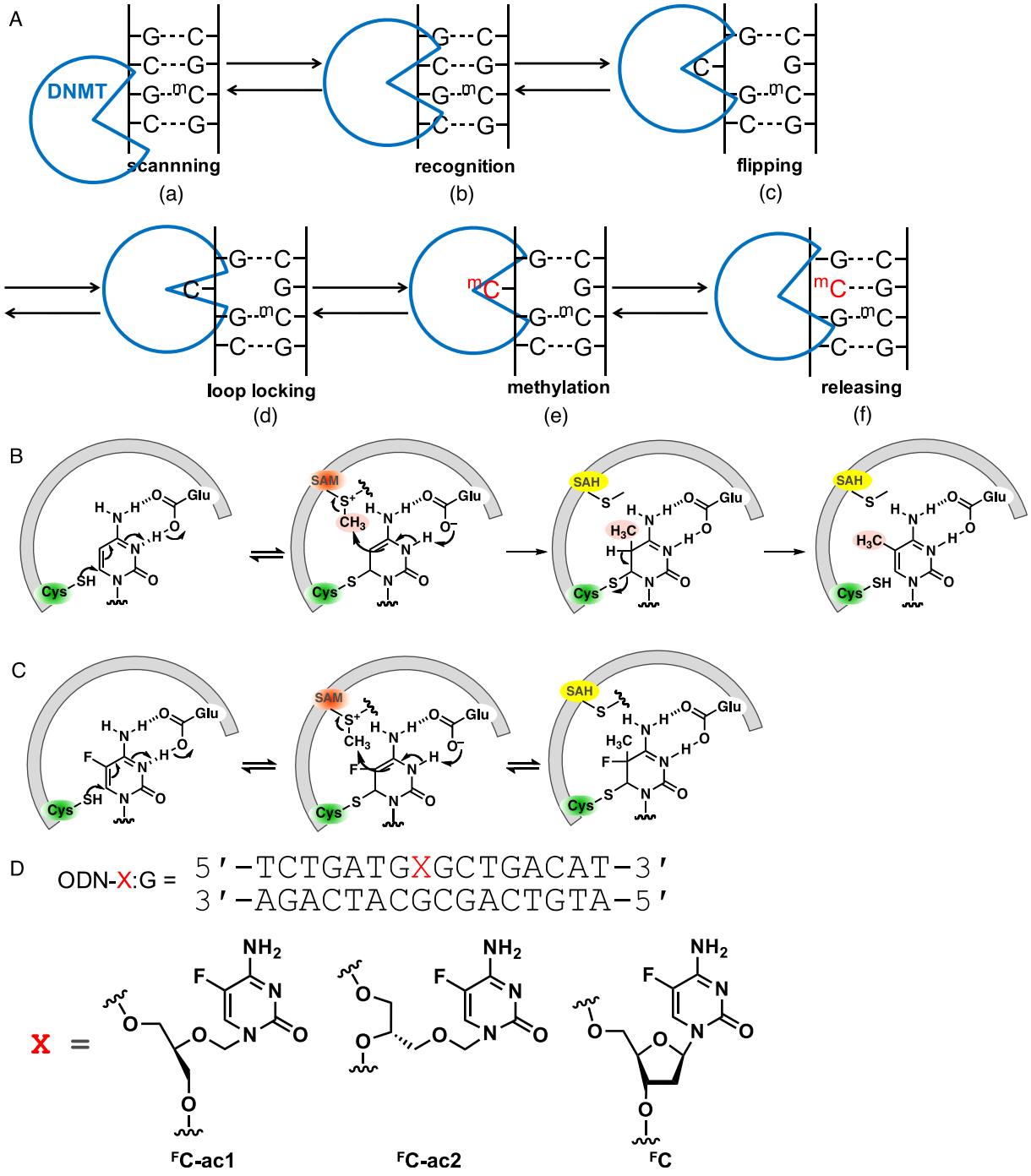

Fig. 1. A) Models for the interactions between DNA and DNMT during methylation reaction. B) Proposed mechanism of cytosine methylation by DNMTs. C) Proposed mechanism of inhibition of DNMTs by 5-fluorocytosine (^{F}C). D) Sequences of synthesized ODNs containing ^{F}C-ac1, ^{F}C-ac2, and ^{F}C.

5-fluorocytosine works as one of the mechanism-based inhibitors of DNMT,[12,13] and the proposed mechanism is illustrated in Fig. 1C. Methylation will occur in the same manner as natural cytosine methylation steps, the releasing step is blocked, and the corresponding intermediate is trapped. It is easy to discuss the complicated reaction steps by its simplification and detect the covalent-bonding ODN-DNMT complex using the difference of the molecular weight by polyacrylamide gel electrophoresis (PAGE) and/or mass spectrum. Therefore, we planned the incorporation of new acyclic nucleosides, which make it easy to flip out from double stranded DNA, into ODNs and investigate the interaction between the ODN and DNMT. Here, we describe the synthesis of new acyclic nucleosides containing 5-fluorocytosine, the interaction between ODNs containing acyclic nucleosides and DNMT, and the physiological properties of modified ODNs. We also discuss the scanning, recognition and flipping mechanism (Fig. 1A(a), (b) and (c)) of DNMT.

Results and discussion

We designed ^{F}C-ac1 and 2 (Fig. 1D) to investigate the recognition by DNMT based on the difference of the glycerol skeleton. ^{F}C-ac1 is a ganciclovir-type 2′-deficient acyclic nucleoside[14], which has the same number of bonds between 5′ and 3′, and a glycerol