Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 343–345

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Selective Reaction to a Flipping Cytidine of the Duplex DNA Mediated by Triple Helix Formation

Fumi Nagatsugi, Daisaku Usui, Takeshi Kawasaki, Minoru Maeda and Shigeki Sasaki*

Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

Received 11 October 2000; accepted 15 November 2000

Abstract—A new nucleoside derivative (**2**) with a butyl spacer between the sugar part and the 2-amino-6-vinylpurine motif has been synthesized. The triplex-forming oligodeoxynucleotide incorporating **2** has achieved strand- and cytidine-selective cross-linking reaction to the G-C target site mediated by triple helix formation. It has been suggested that **2** reacts with a flipping cytidine at the target site.

Gene manipulation at the base level within a specific site of DNA has attracted much attention.[1] Covalent bond formation within duplexes or triplexes has been applied to improve the effectiveness of the antisense or antigene oligonucleotides (ODN).[2] Also, covalent modification of a base theoretically should become a potential chemical method to cause a point mutation that may result in activation or modification of the functional expression of the gene. A recent example has been demonstrated in the study of site-directed mutation with the psoralen-conjugate of the triplex-forming oligonucleotide (TFO).[3] Alkylation of nucleobases with functional TFOs might have potential for such purpose. Previously, oligodeoxynucleotides and related oligomers were conjugated with haloacetyl amide,[4] aryl nitrogen mustard,[5] aziridine units[6] or a minor groove-reactive compound[7] and were used for interstrand cross-linking of a duplex or triplex. In order to develop such reactive oligonucleotides as a useful tool for manipulation of a gene at the base level, it is apparent that further improvement of the selectivity and efficiency of the reactive group is needed. In our own approach to selective functional oligonucleotides, we have developed a 2-amino-6-vinylpurine nucleoside derivative as a cytidine selective cross-linking agent within a duplex.[8,9] Here we wish to report the highly selective reaction to a flipping cytidine of the duplex mediated by triplex formation by using TFO conjugating the new 2-amino-6-vinylpurine derivative.

It has been already demonstrated that the oligonucleotide incorporating 2-amino-6-vinylpurine (**1**) exhibits selective and efficient alkylation to a cytidine at the target site. A further remarkable point of **1** is that the alkylating activity can be auto-generated within the duplex from its stable precursor, a phenylsulfide or phenylsulfoxide derivative (Fig. 1).[10] Proximity effect between the 2-amino-6-vinylpurine motif and a cytidine apparently plays a key role in the selectivity and efficiency of the new cross-linking.

Accordingly, we hypothesized that, if the 2-amino-6-vinylpurine motif of TFO is forced into proximity with a cytidine of a duplex, selective cross-linking should take place. Thus, a new nucleoside derivative (**2**) having a butyl spacer between the sugar part and the 2-amino-6-vinylpurine motif was designed (Fig. 2). The 2-amino-6-vinylpurine motif of **2** would react selectively with the cytidine in the opposite strand when the cytidine is flipped out from a G-C base pair (Fig. 2). Based on a molecular model, the length of the butyl spacer seemed

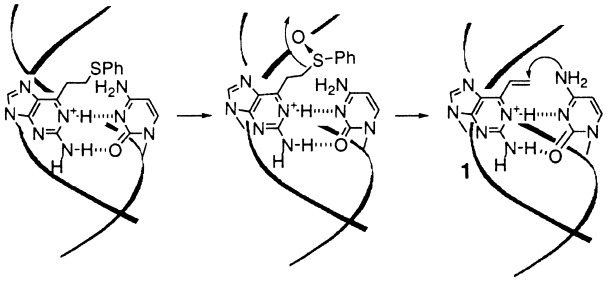


Figure 1. Synchronous activation within duplex.

*Corresponding author. Tel.: +81-92-642-6651; fax: +81-92-642-6654; e-mail: sasaki@phar.kyushu-u.ac.jp

0960-894X/01/$ - see front matter
PII: S0960-894X(00)00666-1

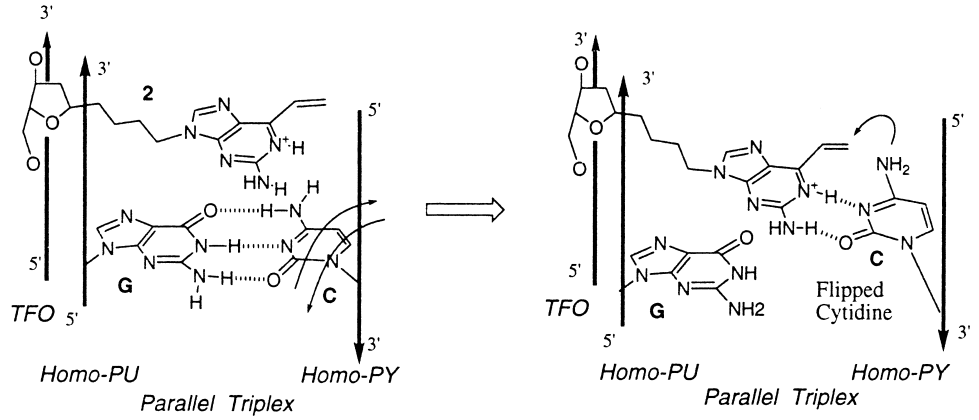
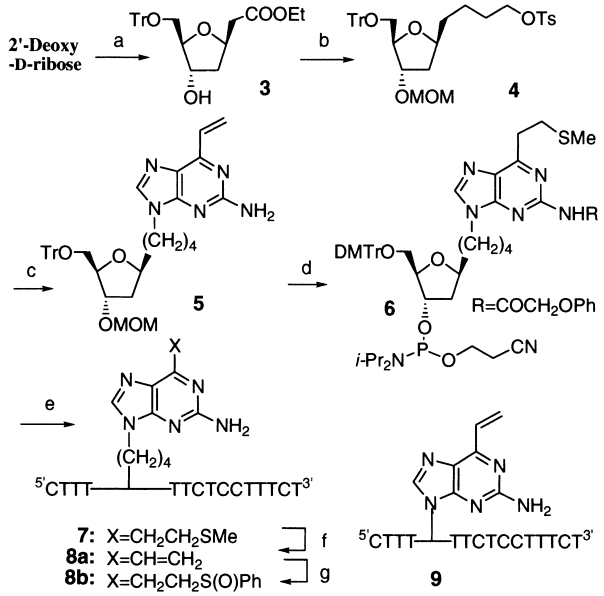


Figure 2. Design of **2** to achieve selective reaction to the flipping cytidine within the triplex.

to be suitable to bring the 2-amino-6-vinylpurine motif into close proximity to the cytidine in the pyrimidine strand of a parallel-type triplex (Fig. 2).

The sugar part **3** was synthesized by β-selective C-glycoside formation via Wittig–Honor–Emmons reaction from 2′-deoxy-D-ribose (Scheme 1).[11] Several transformation reactions with **3** afforded the tosylate (**4**) having the butyl spacer. 9-*N*-Alkylation of 2-amino-6-chloropurine[12] with **4** yielded the desired product as a major isomer, which was used for the Pd(II)-catalyzed cross-coupling reaction with nBu$_3$SnCH=CH$_2$ to afford 2-amino-6-vinylpurine derivative (**5**). After protection of the vinyl group with methylsulfide and the 2-amino group with phenoxyacetyl, the conventional procedure[13] produced the phosphoramidite precursor (**6**) in good yield. The sulfide-protected ODN (**7**) was obtained by applying **6** to an automated DNA synthesizer in good yield after purification with RP-HPLC. The ODN (**7**) was then smoothly converted to **8a** by oxidation with magnesium monoperphthalate (MMPP) following elimination under an alkaline condition.[10] Addition of thiophenol to **8a** and following oxidation with MMPP gave **8b** in good yield. The synthesis of **9** containing **1** was done according to the reported procedure.[10] The structure of ODN was confirmed by UV and MALDI-TOF mass measurements.



Scheme 1. (a) 1) TrCl, DMAP, pyridine, 2) *t*-BuOK, THF, $(EtO)_2P(O)CH_2CO_2Et$, (b) 1) *i*-Pr_2NEt, MOMCl, 2) DIBAL, CH_2Cl_2, 3) NaH, $(EtO)_2P(O)CH_2CO_2Et$, THF, 4) H_2, Pd–C, EtOH, 5) LAH, THF, 6) TsCl, pyridine, (c) 1) 2-amino-6-chloropurine, *t*-BuOK, DMSO, 2) n-$Bu_3SnCH{=}CH_2$, $(Ph_3P)_2PdCl_2$, dioxane, (d) 1) MeSNa, CH_3CN, 2) $PhOCH_2COCl$, 1-HBT, CH_3CN, pyridine, 3) BF_3-Et_2O, Me_2S, 4) DMTrCI, pyridine, 5) *i*-Pr_2NEt, CH_2Cl_2, *i*-$Pr_2NP(Cl)OC_2H_4CN$, (e) 1) automated DNA synthesizer, 2) 28% NH_3, 3) 10% AcOH, (f) 1) 3.0 equiv MMPP, pH 10, 2) 470 mM NaOH, (g) 1) PhSH, 2) 3.0 equiv MMPP, pH 10.

The cross-linking was investigated with the functionalized ODNs (**8a**,**b** or **9**) and the target duplexes (**10**·**11**). In order to clarify the strand of the reaction, one of the two strands (**10** or **11**) labeled with ^{32}P at the 5′-end was used as a tracer. The results were analyzed by gel electrophoresis with 15% denaturing gel and the cross-linked products were identified as slower moving bands relative to the unreacted radiolabeled duplex strand (Fig. 3).

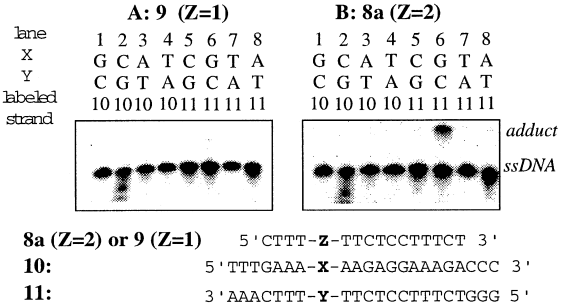


Figure 3. Comparison of the cross-linking reactivity by autoradiogram of gel electrophoresis (15% denaturing gel). **A** with **9** (Z = **1**), **B** with **8a** (Z = **2**). The reaction was done using 10 μM of ODNs (**9**: Z = **1** or **8a**: Z = **2**), 1 μM of target duplex **10**·**11** in a buffer including 10 mM cacodylate, 0.25 mM spermine, 100 mM NaCl, pH 4.5 ± 0.3 at 30 °C. The reaction mixture contained either 5′-^{32}P-labeled **10** or **11** as a tracer. The reaction was stopped by the addition of formamide after 20 h.

It is clearly shown that **1**-bearing ODN (**9**) produces no adducts with any target site (Fig. 3, A). On the other hand, **2**-bearing ODN (**8a**) reacted only to the cytidine within the pyrimidine strand and did not produce any adducts with other target sites (Fig. 3, B, lane 6). All ODN combinations using **7**, **10** and **11** showed triplex melting temperatures higher than 45 °C, indicating that the major part of them are in triplex form under the reaction temperature at 30 °C. Thus, it is clear that the selectivity is not due to difference in triplex stability. These results indicated that **2** exhibited high selectivity to cytidine only at the target site of the pyrimidine strand (**11**) of the duplex, and that the reaction of the 2-amino-6-vinylpurine motif needs precise proximity with the target cytidine. A similar selective reaction to the cytidine in the pyrimidine strand (**11**) was also obtained with the TFO **8b** incorporating the phenylsulfoxide derivative of **2**. Considering that the pyrimidine strand (**11**) contains five cytidines as possible reaction sites, the high selectivity achieved by **2**-bearing ODN (**8a**) is remarkable. The high selectivity is probably ascribed to the complex formation between **2** and the flipping-cytidine as we anticipated in the molecular design of **2**. This speculation has been supported by the fact that higher yields of the cross-linking reactions were obtained with duplexes containing the mismatch pairs (X–Y in **10•11**: A–C or T–C), in which a higher ratio of the flipping-cytidine was expected compared to a matched G–C pair. That is, the yield of cross-linked products increased from 25% for G–C (a match pair) to 40% for A–C and T–C (a mismatch pair). As it is clear from the estimation with the electrophoresis using nature gel that the duplex containing a mismatch pair of A–C or T–C is only slightly less stable than that with a G–C pair at room temperature, increase in the cross-linking yield may be attributable to a higher ratio of the flipping cytidine of these mismatch pairs. In contrast, lower cross-linking yield of 16% was obtained at the C–C mismatch site probably due to thermal instability of the duplex containing the C–C mismatch as evidenced by the electrophoresis.[14]

In conclusion, we have successfully demonstrated a highly selective cross-linking reaction to a flipping cytidine within the triplex using TFO bearing 2-amino-6-vinylpurine derivatives (**2**). The selective reactivity of **2** should be useful in application to site-directed modification of a cytidine within a selected target. Further work is now under way in the search for the appropriate structure having higher reactivity in the triplex forming cross-linking reaction.

Acknowledgements

The authors are grateful for support by Grant-in-Aid for Scientific Research on Priority Area (A), Area No. 404 to SS and for Young Scientist to FN from the Ministry of Education, Science, Sports and Culture, Japan.

References

1. Woolf, T. M. *Nature Biotech.* **1998**, *16*, 341 and references cited therein.
2. References cited in a review, Herdewijn, P. *Antisense & Nucleic Acid Drug Development* **2000**, *10*, 297.
3. (a) Wang, G.; Lwvy, D. D.; Seidman, M. M.; Glazer, P. M. *Mol. Cell. Biol.* **1995**, *15*, 1759. (b) Faruqi, A. F.; Krawczyk, S. H.; Matteucci, M. D.; Glazer, P. M. *Nucleic Acids Res.* **1997**, *25*, 633.
4. (a) Povsic, T. J.; Strobel, S. A.; Dervan, P. B. *J. Am. Chem. Soc.* **1992**, *114*, 5934. (b) Povsic, T. J.; Dervan, P. B. *J. Am. Chem. Soc.* **1990**, *112*, 9428. (c) Grant, K. B.; Dervan, P. B. *Biochemistry* **1996**, *35*, 12313.
5. (a) Reed, M. W.; Lukhtanov, E. A.; Gorn, V.; Kutyvin, I.; Gall, A.; Wald, A.; Meyer, R. B. *Bioconjugate Chem.* **1998**, *9*, 64. (b) Belousov, E. S.; Ahonina, I. A.; Podyminogin, M. A.; Gamper, H. B.; Reed, M. W.; Wydro, R. M.; Meyer, R. B. *Nucleic Acids Res.* **1997**, *25*, 3440.
6. (a) Reed, M. W.; Wald, A.; Meyer, R. B. *J. Am. Chem. Soc.* **1998**, *120*, 9729. (b) Shaw, J.-P.; Milligan, J. F.; Krawczyk, S. H.; Matteucci, M. *J. Am. Chem. Soc.* **1991**, *113*, 7765.
7. Lukhtanov, E. A.; Mills, A. G.; Kutyavin, I. V.; Gorn, V. V.; Reed, M. W.; Meyer, R. B. *Nucleic Acids Res.* **1997**, *25*, 5077.
8. (a) Nagatsugi, F.; Uemura, K.; Nakashima, S.; Maeda, M.; Sasaki, S. *Tetrahedron Lett.* **1995**, *36*, 421. (b) Nagatsugi, F.; Uemura, K.; Nakashima, S.; Maeda, M.; Sasaki, S. *Tetrahedron* **1997**, *53*, 3035.
9. (a) Kawasaki, T.; Nagatsugi, F.; Maeda, M.; Sasaki, S. *Nucleic Acids Symposium Series* **1999**, *42*, 43. (b) Nagatsugi, F.; Kawasaki, T.; Tanaka, Y.; Maeda, M.; Sasaki, S. *Nucleic Acids Symposium Series* **1998**, *39*, 103. c) Nagatsugi, F.; Kawasaki, T.; Maeda, M.; Sasaki, S. *Nucleic Acids Symposium Series* **1997**, *37*, 67.
10. Nagatsugi, F.; Kawasaki, T.; Usui, D.; Maeda, M.; Sasaki, S. *J. Am. Chem. Soc.* **1999**, *121*, 6753.
11. Matsuura, N.; Yashiki, Y.; Nakashima, S.; Maeda, M.; Sasaki, S. *Heterocycles* **1999**, *51*, 975.
12. Kjellberg, J.; Liljenberg, M.; Johansson, N. G. *Tetrahedron* **1986**, *42*, 6541.
13. Nagai, H.; Fujiwara, T.; Fujii, M.; Sekine, T.; Hata, T. *Nucleic Acids Res.* **1989**, *21*, 8581.
14. This accords with the reported fact, Peyret, N.; Seneviratne, P. A.; Allawi, H. T.; SantaLucia, J., Jr. *Biochemistry* **1999**, *38*, 3468.