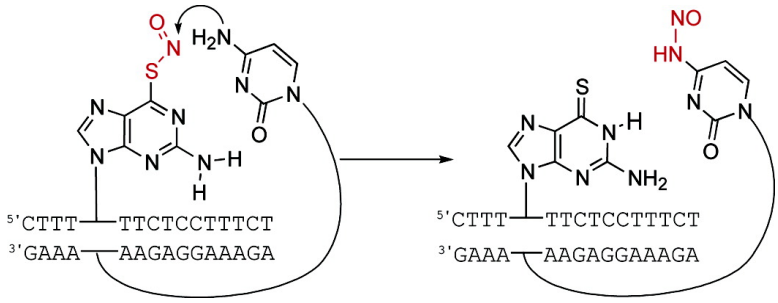
Communication

Sequence- and Base-Specific Delivery of Nitric Oxide to Cytidine and 5-Methylcytidine Leading to Efficient Deamination

Md. Monsur Ali, Md. Rowshon Alam, Takeshi Kawasaki, Shizuka Nakayama, Fumi Nagatsugi, and Shigeki Sasaki

J. Am. Chem. Soc., **2004**, 126 (29), 8864-8865• DOI: 10.1021/ja0498888 • Publication Date (Web): 03 July 2004



More About This Article

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

- Supporting Information
- 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 07/03/2004

Sequence- and Base-Specific Delivery of Nitric Oxide to Cytidine and 5-Methylcytidine Leading to Efficient Deamination

Md. Monsur Ali,† Md. Rowshon Alam,† Takeshi Kawasaki, Shizuka Nakayama,† Fumi Nagatsugi,† and Shigeki Sasaki*,†

Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan, and CREST, Japan Science and Technology Agency, Kawaguchi Center Building, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan

Received January 8, 2004; E-mail: sasaki@phar.kyushu-u.ac.jp

Nitric oxide (NO) is an important endogenous regulatory molecule,[1] and *S*-nitrosothiols are believed to play a significant role in NO storage, transport, and delivery.[2] On the basis of their ability to generate NO in vivo, *S*-nitrosothiols can be used as therapeutic drugs.[3] Conversely, NO and its derivatives are suspected of having cytotoxic, carcinogenic, and mutagenic properties.[4] A number of theoretical and experimental studies have assessed the damage to DNA by NO and its derivatives and have suggested that NO-induced mutagenesis arises from the initial nitrosation of the amino group of nucleobases, followed by deamination.[5] While studying specific reactions with DNA, we became interested in whether or not sequence-specific delivery of NO to a specific site of DNA followed by specific deamination would be possible. Such a method would be useful to better understand NO-induced DNA damage. Furthermore, if NO-induced deamination of a target nucleobase could be controlled, it would become an innovative biological tool for manipulating a gene at a single nucleobase level.[6] In this study, we have established an innovative method for the highly efficient and selective delivery of NO to cytidine and 5-methylcytidine in a sequence-specific method.

We designed a NO-transfer reaction from *S*-nitroso thioguanine to an imino tautomer of cytosine (Figure 1). Ab initio calculations suggested that NO-transfer would proceed with low activation energy, accompanied by hydrogen transfer from N3 of cytosine to N1 of thioguanine.[7] We also expected the proximity effect within the duplex to be beneficial for NO transfer.

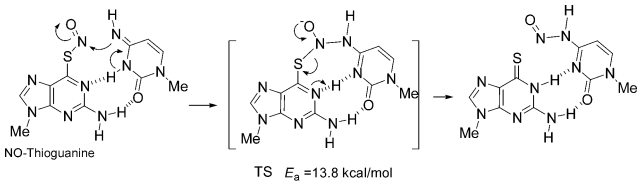


Figure 1. Design of *S*- to *N*-nitroso transfer.

The thioguanosine derivative was synthesized and incorporated into ODN **1** according to the methods reported previously.[8] Nitrosation of the thioguanosine-containing ODN **1** was carried out by a NO transfer reaction with *S*-nitroso-*N*-acetylpenicillamine (SNAP) under alkaline conditions at pH 10.[9] The reaction progress was followed by HPLC, and a new peak **2** was isolated (Figure 2). The nitrosyl group of the isolated peak **2** was identified by a method established by the Nagano group, in which the diaminofluorescein derivative (DAF2) reacts with the NO^+ species to generate fluorescent DAF2-triazole.[10−14]

It should be noted that the nitrosylated ODN **2** exhibits high stability with a half-life of about 1 week at room temperature at pH 7. The same nitrosation reaction of the monomer thioguanosine with SNAP produced no nitrosylated product but the disulfides of thioguanosine and SNAP, indicating that the high stability of the nitrosyl group of ODN **2** is attributable to its incorporation into the ODN.[15]

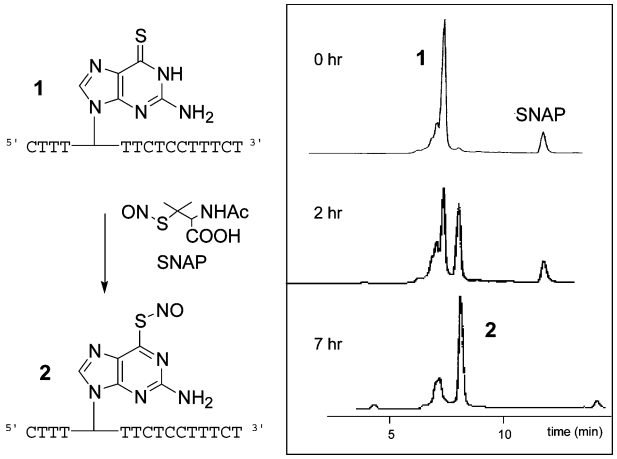


Figure 2. Nitrosation of thioguanosine-bearing ODN. The reaction was carried out using 0.3 mM **1** and 6 mM SNAP in carbonate buffer at pH 10 at room temperature. The reaction mixture was analyzed by HPLC (ODS column, 1 mL/min; solvent A = 0.1 M TEAA buffer; solvent B = CH_3CN, linear gradient from 10 to 30% over 20 min, 40% for 30 min; monitored at 260 nm). See also Figures 7 and 9 in Supporting Information.

An interstrand NO-transfer reaction was carried out using ODN **2** and its complementary ODN **3** (X = dC or d^mC) and was followed by HPLC (Figure 3). Figure 3A illustrates the HPLC change of the reaction between **2** and **3** (X = d^mC), and its conversion yields from **2** to **1** are plotted in Figure 3B. A similar conversion curve of **2** to **1** was obtained with ODN **3** with dC. As **3** and **4** could not be separated by HPLC, the peak containing a mixture of **3** and **4** was isolated, and their nitrosyl species were analyzed with DAF2. The increase of DAF2-triazole obtained from the isolated peak including **3** and **4** correlates well with the conversion of **2** to **1**.[13,14] Almost the same amounts of DAF2-triazole were detected from the initial ODN **2** and the product ODN **4** (X = dC or d^mC) isolated after 8 h, indicating that NO transfer from **2** to **3** (X = dC or d^mC) occurred in almost quantitative yields.[13] These results with DAF2 show that the conversion of **2** to **1** corresponds to NO transfer reaction.

In contrast to the rapid reaction of **2** with dC or d^mC-bearing ODN **3**, a transfer reaction was not observed either with ODN **3** having dT, dA, or dG at the target site or with ODN **5**−**7** having dC at a nontarget site (Figure 3B).[17] Glutathione did not react with **2** even at high concentrations (1 mM). These results indicate that the highly selective reactivity of **2** is due to the efficient proximity

† Japan Science and Technology Agency.

10.1021/ja0498888 CCC: $27.50

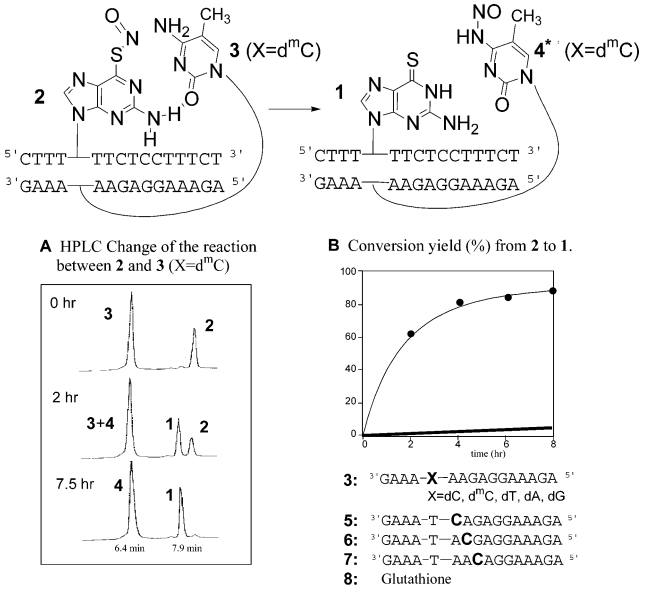


Figure 3. NO transfer reaction with the use of ODN **2**. The asterisk (*) indicates that a representative tautomer is shown. A: The reaction was carried out using 10 μM each of **2** and ODN **3**, **5**−**7**, or 1 mM glutathione in 50 mM MES buffer containing 100 mM NaCl at pH 7 at 25 °C, and followed by HPLC. B: Closed circles and curve correspond to the reaction with **3** (X = dC or d^{m}C); the thick line corresponds to the reaction with **3** (X = dT, dA, or dG) or **5**−**8**.

effect between the *S*-NO thioguanosine and the target dC and d^{m}C in the duplex.[17]

Since nitrosylation of an amino group of a nucleoside is reported to cause deamination,[5] we next analyzed deaminated products. Specific deamination of dC and d^{m}C, i.e., the formation of dU and dT, respectively, imply specific nitrosylation of their 4-amino group. After the NO-transfer reaction with ODN **3** (X = dC) was completed, the mixture was kept at pH 5 for 4 days, and then ODN **4** (X = dC) was isolated and subjected to enzymatic digestion with BAP and VPDE. HPLC analysis of the hydrolysates showed peaks of dU (8%) and the dC-diazoate[18] (14%) together with dG and dA. Similarly, ODN **4** (X = d^{m}C) was isolated from the reaction mixture of NO-transfer with ODN **3** (X = d^{m}C), but the mixture was kept at pH 5 for 1 day in the presence of $CaCl_2$. It should be noted that the transformation ratio from d^{m}C to dT was as high as 42% together with d^{m}C-diazoate (13%)[19] (Figure 4B). Since other deaminated products were not observed in Figure 4B, it has been clearly demonstrated that NO is transferred selectively to 4-NH_2 of d^{m}C.

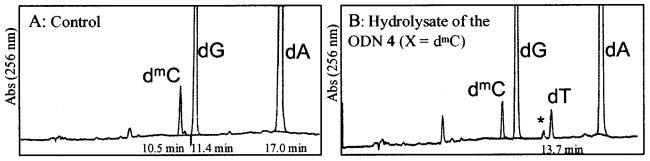


Figure 4. Detection of thymidine from 5-methylcytidine. A: The control experiment was carried out using ODN **1** and the target ODN **3** and analyzed as described in B. B: After 8 h, the NO transfer mixture was kept at pH 5 in the presence of 1 mM $CaCl_2$. The compound producing peak **4** in Figure 3A was isolated, hydrolyzed with BAP and VPDE, and analyzed by HPLC. The peak marked with an asterisk corresponds to d^{m}C-diazoate.[19]

The samples of ODN **4** isolated after treatment under acidic conditions were analyzed with DAF2, decreasing the NO level to about 80% for X = dC and to about 50% for X = d^{m}C compared to those analyzed immediately after the transfer reaction. These results indicate that the remaining NO species in ODN **4** spontaneously return to dC or d^{m}C when the ODN is hydrolyzed to nucleosides. These findings agree with the known fact that nitrosylated species of 4-NH_2 of the monomer dC and d^{m}C are so unstable that they predominantly return to dC and d^{m}C together with the deaminated products and the corresponding diazotes.[18,19] Retention of NO species in DNA **4** for a long time in solution exhibits remarkable contrast to its monomer chemistry. A detailed study is being conducted to determine the exact structure of DNA **4**.

In conclusion, we have demonstrated the first example of sequence- and base-specific delivery of nitric oxide to cytidine and 5-methylcytidine. The selectivity and efficiency of NO transfer followed by deamination exhibited in this study are extremely high compared to the conventional methods of using NO gas or other nitrosating agents. The innovative method described in this study would be useful to better understand the role of NO in DNA damage. Applications of this new NO delivery method to selective induction of point mutation at the translation or polymerization steps are now ongoing.

Acknowledgment. This work was supported by a Grant-in-Aid for Scientific Research (B) from Japan Society for the Promotion of Science (JSPS), CREST from Japan Science and Technology Agency, and Kyushu University P&P "Green Chemistry" Program. We also acknowledge Professor Kenji Hori at Yamaguchi University, Japan, for ab initio calculation.

Supporting Information Available: UV spectra for pK_a measurements and figures for analysis of the NO transfer reaction by HPLC chromatography and by DAF2 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Moncada, S.; Palmer, R. M.; Higgs, E. A. *Pharmacol. Rev.* **1991**, *43*, 109−142.
(2) Williams, D. L. H. *Acc. Chem. Res*. **1999**, *32*, 869−876.
(3) Al-Sa'doni, H.; Ferro, A. *Clin. Sci.* **2000**, *98*, 507−520.
(4) Lala, P. K.; Chakraborty, C. *Lancet Oncol.* **2001**, *2*, 149−156.
(5) Burney, S.; Caulfield, J. L.; Niles, J. C.; Wishnok, J. S.; Tannenbaum, S. R. *Mutat. Res.* **1999**, *424*, 37−49.
(6) Recent examples: (a) Seidman, M. M.; Glazer, P. M. *J. Clin. Invest.* **2003**, *112*, 487−494. (b) Zhou, Q. B.; Rokita, S. E., *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 15452−15457. (c) Sasaki, S. *Eur. J. Pharm. Sci*. **2001**, *13*, 43−51 and references therein.
(7) An ab initio caluculation was done at the B3LYP/6-31G* level to produce a transition state with an activation energy of 13.8 kcal/mol.
(8) Kadokura, M.; Wada, T.; Seio K.; Sekine, M. *J. Org. Chem.* **2000**, *65*, 5104−5113. See also references on thionucleosides cited therein.
(9) Wang, K.; Wen, Z.; Zhang, W.; Xian, M.; Cheng, J. P.; Wang, P. G. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 433−436.
(10) Kojima, H.; Nakatsubo, N.; Kikuchi, K.; Kawahara, S.; Kirino, Y.; Nagoshi, H.; Hirata, Y.; Nagano, T. *Anal. Chem.* **1998**, *70*, 2446−2453.
(11) Nakatsubo, N.; Kojima, H.; Kikuchi, K.; Nagoshi, H.; Hirata, Y.; Maeda D, Imai, Y.; Irimura, T.; Nagano, T. *FEBS Lett.* **1998**, *427*, 263−266.
(12) Li, H.; Meininger, C. J.; Wu, G. *J. Chromatogr. B* **2000**, *746*, 199−207.
(13) See Figures 7 and 9 in Supporting Information.
(14) Nitrosyl groups of **2** and **4** have been detected by negative-ion ESI-TOF MS measurements, although we have also observed fragmentation peaks due to cleavage of the *S*-NO and *N*-NO bonds. MS (*m*/*e*): [ODN **1**]$^{4-}$ found 1192.22, calcd 1192.19; [ODN **2**]$^{4-}$ found 1200.07, calcd 1199.94; [ODN **3** (X = dC)]$^{3-}$ found 1668.18, calcd 1668.31; [ODN **3** (X = d^{m}C)]$^{3-}$ found 1673.21, calcd 1672.98; [ODN **4** (X = dC) − H + K$^+$]$^{4-}$ found 1267.64, calcd 1267.95; [ODN **4** (X = d^{m}C) − H + Na$^+$]$^{3-}$ found 1690.31, calcd 1689.97.
(15) The higher pK_a of the thioguanosine in ODN **1** than in its monomer[16] may be responsible for the stability of the nitrosyl group in ODN **2**, since thiols with higher pK_a values tend to produce more stable *S*-NO bonds: Lu, J. M.; Wittbrodt, J. M.; Wang, K.; Wen, Z.; Schlegel, H. B.; Wang, P. G.; Cheng, J. P. *J. Am. Chem. Soc.* **2001**, *123*, 2903−2904.
(16) Figure 5 in Supporting Information.
(17) Progress of NO transfer reaction and the detection of DAF-2 triazole are illustrated in Figures 6−9 in Supporting Information.
(18) Suzuki, T.; Nakamura, T.; Yamada, M.; Ide, H.; Kanaori, K.; Tajima K.; Morii, T.; Makino, K. *Biochemistry* **1999**, *38*, 7151−7158.
(19) Suzuki, T.; Yamada, M.; Nakamura, T.; Ide, H.; Kanaori, K.; Tajima, K.; Morii, T.; Makino K. *Bioorg. Med. Chem.* **2002**, *10*, 1063−1067.

JA0498888