NO induced novel DNA lesions: Formation mechanism

Masaki Yamada, Toshinori Suzuki, Kenji Kanaori¹, Kunihiko Tajima¹, Takashi Morii and Keisuke Makino
Institute of Advanced Energy, Kyoto University, Gokasho, Uji, Kyoto 611-0011, Japan and ¹Department of Applied Biology, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan

ABSTRACT

2'-Deoxyguanosine was treated with NO/O₂ mixture at pH 7.0~7.8, and as well as the known major products such as 2'-deoxyxanthosine and 2'-deoxyoxanosine, some unidentified products were detected by RP-HPLC. In the present study, one of them has been characterized as a novel lesion, N²-nitro-2'-deoxyguanosine by spectrometric and chromatographic analysis. The mechanism for the production is also discussed.

Introduction

Nitric Oxide (NO) plays physiologically important roles. On the other hand, NO also reacts with oxygen (O₂) to generate reactive nitrogen species, which may be damaging to nucleobases to cause mutagenesity or genetic diseases.

It has been reported that NO induces deamination of DNA under aerobic condition¹: In the case of 2'-deoxyguanosine (dGuo), 2'-deoxyxanthosine (dXao) is generated as a major deamination product. We have reported that 2'-deoxyoxanosine (dOxo) is also produced from dGuo as an NO-induced DNA lesion². It has been elucidated that the N-glycosidic bond of dOxo is as stable as that of dGuo and hydrolyzed 44-fold more slowly than that of dXao³, and that in the DNA polymerase chain elongation, dOxo 5'-triphosphate is incorporated not only opposite 2'-deoxycytidine moiety on the template but also opposite thymidine moiety⁴. Furthermore dOxo has been found to react efficiently with amino groups, resulting in formation of cross-link adducts with amino acids⁵. These results imply that the formation of dOxo in DNA or nucleotide pool can cause mutagenesis or carcinogenesis.

In the present study, in order to investigate NO-induced DNA lesions in more detail, we have focused on the effect of O₂ ratio on the NO-dGuo reaction. dGuo was subjected to the reaction by changing the O₂ ratio in NO/O₂ mixture.

Here we report the characterization of the product and its formation mechanism.

Results and Discussion

When we treated 10 mM dGuo in 100 mM phosphate buffer (pH 7.4) with NO/O₂ (0.5/1.5 mL/sec) mixture gas at pH 7.0~7.8, in addition to dXao or dOxo, some unidentified products (referred to as Peak 1 and 2) were observed by RP-HPLC (Figure 1). Peak 1 has been identified as nitro or nitrosooxy derivative of guanine on the basis of LCMS (m/z = 195) and UV (λ_max = 380 nm) spectra⁶. Peak 2 (λ_max = 330 nm) was isolated by preparative RP-HPLC and the
structure was characterized. 'H-NMR spectrum of Peak 2 (in DMSO-\(d_6\)) showed a set of signals of 2'-deoxyribose moiety, imino proton, and 8H. Negative ion APCI-LCMS analysis showed nitro or nitrosooxy group specific fragment pattern: m/z = 311 [M\(^-\)], 195 [M_{base}\(^-\)], 166 and 150. Peak 2 was reduced by sodium borohydride to 2-hydradino-2'-deoxyinosine. This result indicates that Peak 2 has a nitro group. On the basis of these data, we have identified the product as \(N^2\)-nitro-2'-deoxyguanosine (\(N^2\)-NO\(_2\)-dGuo) (Figure 2). The yield of \(N^2\)-NO\(_2\)-dGuo was increased with increase in the \(O_2\) ratio and pH.

The formation mechanism of \(N^2\)-NO\(_2\)-dGuo also has been investigated. Treatments of dGuo with gaseous NO\(_2\) and tetranitromethane [nitronium ion (NO\(_2^+\)) generation reagent] did not yield \(N^2\)-NO\(_2\)-dGuo. This allowed to rule out direct attack of NO\(_2\) radical and NO\(_2^+\) toward amino group of dGuo. We now speculate that \(N^2\)-NO\(_2\)-dGuo is generated by the modified mechanism adopted to the formation of dOxo and dXao.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** RP-HPLC chromatogram for dGuo treated with NO/\(O_2\) mixture. dGuo was dissolved at 10 mM in 10 ml of 100 mM phosphate buffer(pH 7.4) and NO\(_2\) (0.5/1.5 mL/sec) was bubbled through a grass frit into the stirring solution at 37 °C. An HPLC system equipped with an ULTRON ODS column (6 x 150 mm) was used. The eluent was 100 mM formic acid and the flow rate 1.7 mL/min. The CH\(_3\)CN concentration was increased from 0% to 20% for 20 min in the linear gradient mode.

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Structure of \(N^2\)-nitro-2'-deoxyguanosine.

**References**