Formation of 8-nitroguanine from 2'-deoxyguanosine by NO/O_2 system

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ABSTRACT

We investigated the reaction of 2'-deoxyguanosine (dGuo) with NO/O_2 gas mixture under physiological condition and detected 8-nitroguanine, which is known as a novel DNA lesion caused by peroxynitrite (ONOO⁻). The yield increased with increase in the ratio of O_2 and pH. The reaction mechanism is discussed.

INTRODUCTION

NO produced in vivo has many physiological functions such as vasodilator and neurotransmission and also has toxic effects such as DNA damage. Wink et al.¹ have shown that NO reacts with DNA in the presence of O_2 to induce oxidative deamination.

Recently we have reported that 2'-deoxyoxanosine (dOxo)² is produced from NO-treated dGuo under aerobic condition in addition to deamination product, 2'-deoxyxanthosine (dXao). Since NO itself cannot induce deamination of dGuo, O_2 should be a crucial cofactor for damaging DNA.

Goldstein et al.³ proposed NO autoxidation scheme (Scheme 1) in which there are several reactive intermediates with potential cytotoxic and mutagenic properties. In the scheme, they suggested that not only N_2O_3, which is known as deaminating agent, but the other reactive intermediates are important for its toxic effect.

In order to explore which species reacts with dGuo and what is produced, we examined the reaction of dGuo by changing the ratio of NO/O_2.

\[
\text{NO} + \text{O}_2 \rightleftharpoons \text{ONOO} \quad (1) \\
\text{ONOO} + \text{NO} \rightarrow \text{ONOONO} \quad (2) \\
\text{ONOONO} \rightarrow 2\text{NO}_2 \quad (3) \\
\text{NO}_2 + \text{NO} \rightleftharpoons \text{N}_2\text{O}_3 \quad (4) \\
\text{N}_2\text{O}_3 + \text{H}_2\text{O} \rightarrow 2\text{NO}_2^- + 2\text{H}^+ \quad (5)
\]

Scheme 1. Autoxidation of NO in aqueous solution.

MATERIALS AND METHODS

The NO bubbling system used was previously described. In physiological condition, 10 mM dGuo was dissolved in 10 ml of phosphate buffer (0.1 M, pH 7.4). dGuo was also dissolved in 50 mM NaOH (pH~12) or 50 mM HCl solution (pH~1). NO/O_2 gas mixture was kept bubbling through a grass frit into the stirred solution at 37°C. Authentic 8-nitroguanine was kindly gifted by Dr. Hiroshi Ohshima of IARC, France.
RESULTS AND DISCUSSION

NO/O2 mixture (flow rates (ml/s) being 0.5/1.5) was bubbled into dGuo solutions at various pHs. At pH 7.4, a large amount of dXao, a small amount of dOxo and several unknown products were detected by RPHPLC. One of these unknown products was identified as 8-nitroguanine (Figure 1) by comparison of the UV spectrum (λmax = 400 nm) and the RPHPLC retention time with those of an authentic sample. NMR and IR spectra supported the identification. When only NO was bubbled into dGuo solution, 8-nitroguanine was not detected\(^4\). In the presence of O2, production of 8-nitroguanine increased with increase of O2. Under alkaline conditions, the amount increased while under acidic conditions, it was almost equal to that obtained at pH 7.4.

![Figure 1. Structure of 8-nitroguanine.](image)

8-Nitroguanine has been identified previously as a DNA damage produced by ONOO\(^-\), however the mechanism and reactive species for this production have not been elucidated yet. In our current system, among the species in Scheme 1, ONOO\(_2\), NO2, and N\(_2\)O\(_3\) are known to be reactive (Note that ONOO has low reactivity\(^3\)). N\(_2\)O\(_3\) does not act as a nitrating agent. So this species can be ruled out, indicating that the nitrating agent must be one or both of the other two species. We examined the reaction of dGuo with NO\(_2\), however, 8-nitroguanine was not observed, suggesting that ONOO\(_2\) is the most possible nitrating agent. In ONOO\(^-\) reaction, Yermilov et al\(^6\) proposed that NO2, NO2\(^+\) and trans-ONOONO could be reactive species for the production of 8-nitroguanine. Thus NO2\(^+\) or trans-ONOONO may be formed from ONOO\(_2\) in NO/O2 system.

Conclusively we elucidated that 8-nitroguanine is produced in the presence of NO/O2. More detailed mechanism for the production of 8-nitroguanine will be discussed.

REFERENCES