Reactions of NO with Nucleic Acid Bases and Their Biological Implication

K. Makino
International Innovation Center and Institute of Advanced Energy, Kyoto University

NO is generated in human bodies and physiologically active.

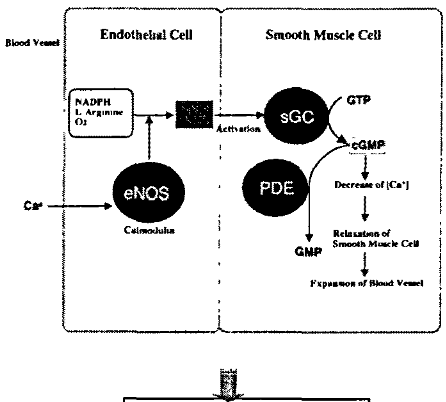
Activation of *soluble* Guanylate Cyclase - Release of cGMP (2nd Messenger):

- **Relaxation of blood vessel smooth muscle**
- **Relaxation of trabecular smooth muscle - Penile erection**
- **Memory formation - Long-term potentiation (LTP)**
- **Neuronal transmission**
- **Inhibition of leukocyte adhersion**
- **Inhibition of platelet aggregation**

Chemical Reactivity and Free Radical Nature:

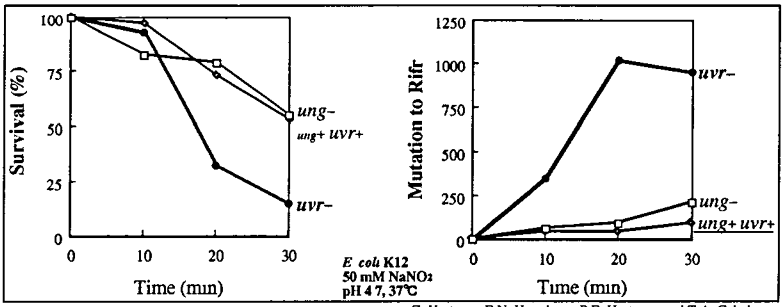
- **Cytotoxicity - Peroxynitrite ($NO + O2^{-} \rightarrow ONOO^{-}$) formation in phagocytosis**
- **ADP-ribosylation of G-proteins and GAP dehydrogenase**
- **Alteration of Iron-Sulfur Clusters**
  - **-Inhibition of ribonucleotide reductase**
  - **-Inhibition of aconitase, CoQ oxidoreductases**

Example: Relaxation of Blood Vessel Smooth Muscle



Inflammation ~~overproduces NO. What ha~~ppens?

Effects of Defects in DNA Repair on the Survival and Mutation of *E.coli* Cultured in HNO2-Containing Medium



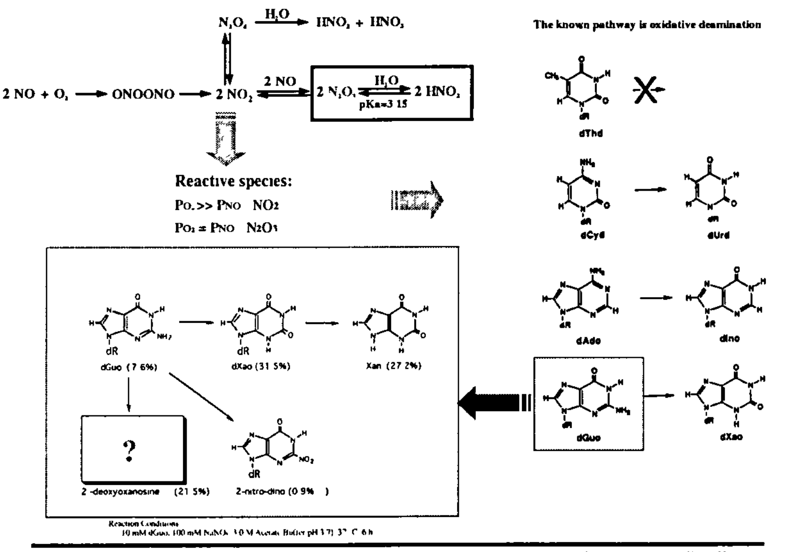
Z Hartman, E N Henrikson, P E Hartman, and T A Cebula
Environmental and Molecular Mutagenesis, 24, 168-175 (1994)

Above data suggest that cross-links may take place between DNA and compounds in nuclei
Nitrous acid mutagenesis is enhanced by spermidine, indicative of cross-links
G/C→A/T conversion is also responsible for the mutagenesis

Subject 1: Mechanism for the cross-links.
Subject 2: Mechanism for G/C→A/T conversion.

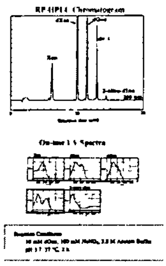
International Innovation Center Kyoto University

Generation of Reactive Species from NO and Their Known Reactions with DNA

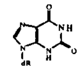


International Innovation Center, Kyoto University

Product Analysis

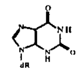


Elemental Analysis
Anal ($C_{10}H_{12}N_4O_5$)
Calcd C, 44.78; H, 4.51; N, 20.89
Found C, 44.75, H, 4.49, N, 20.90

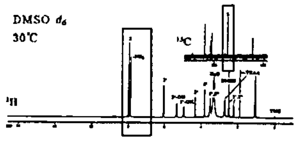


dXao

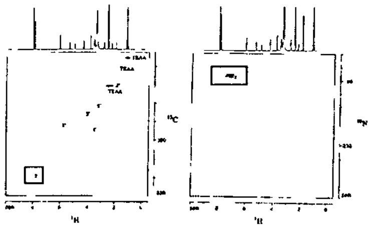
MS Analysis
HRMS MASS ($C_5H_4N_4O_2$)
Calcd 152.03340
Found 152.03249



Xan

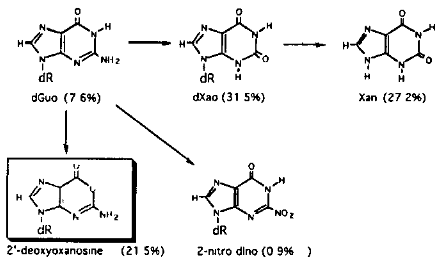


In the ring, there are five carbons and two protons.



One proton is on C, and the other one exchangeable on N

Missing Product is 2'-Deoxyoxanosine



Reaction Conditions
10 mM dGuo 100 mM NaNO$_2$ 3.0 M Acetate Buffer (pH 3.7) 37°C 6 h

Oxa was found in 1981.

Oxanosine was isolated as a novel antibiotic in 1981 from the culture broth of *Streptomyces capreolus* MG265-CF3 [1]

Oxanosine had weak antibacterial activity against *E. coli* K 12 and *Proteus mirabilis* IFM OM 9 [1]

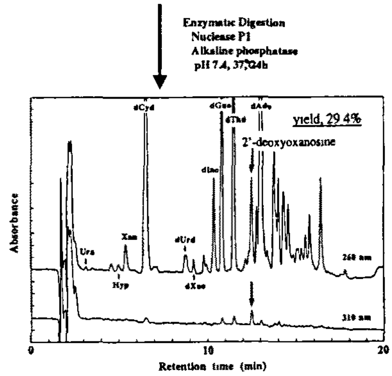
Oxanosine also inhibited the growth of HeLa cells in culture and induced reversion toward the normal phenotype of K-*ras*-transformed rat kidney cells [2]

2'-Deoxyoxanosine was synthsized from oxanosine and exhibited a stronger antineoplastic activity than oxanosine [3]

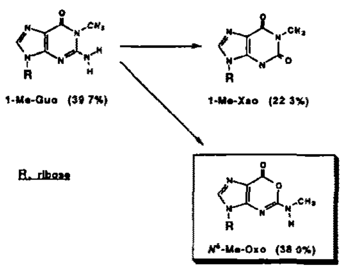
(1) Shimada et al. (1981) *J. Antibiot.* 34, 1216–1218
(2) Itoh et al. (1989) *Cancer Res.*, 49 996–1000.
(3) Kato et al. (1984) *J. Antibiot.*, 37 941–942

Calf Thymus DNA + HNO_2

Reaction conditions
1 mg/mL Calf thymus DNA, 100 mM NaNO2
3 0 M Acetate buffer, pH 3 7, 37C, 6 h

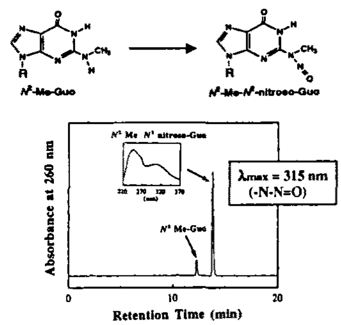


Reaction Mechanism: First Attack and the Product



Reaction Conditions
1 mM 1-Me-Guo, 100 mM $NaNO_2$, 3 0 M Acetate Buffer
pH 3 7, 37 °C, 6 h

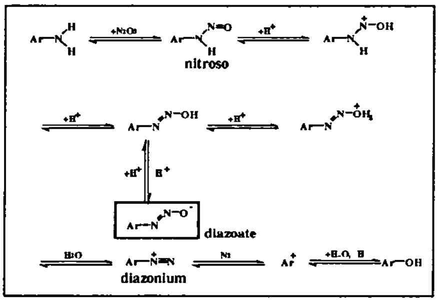
The reaction starts with the attack to the exo-NH_2



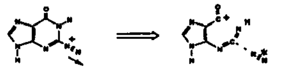
Reaction conditions
10 mM N^2-Me-Guo, 100 mM $NaNO_2$, 3.0 M Acetate buffer
pH 3 7, 37C, 2h

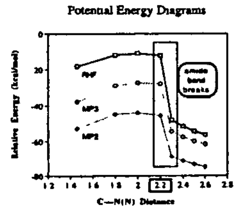
By the attack, nitrosoamine is formed.

Reaction Mechanism: Following Reactions



-NH-NO is converted to -N⁺-N=O or -N=N-O⁻.

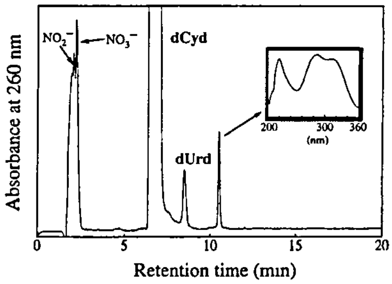


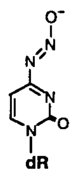


(1) Glaser R Son, M S J Am Chem Soc 1996, 118 10942-10943

Diazonium is produced and the ring-opening follows.

Diazoate Formation from dCyd in HNO_2



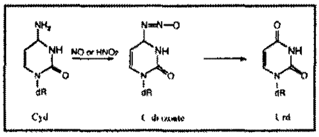


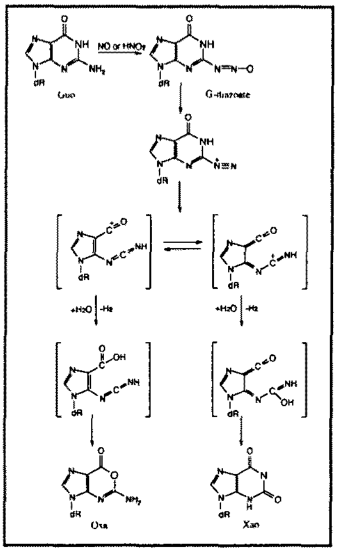
compound 1
(FW=255)

Reaction conditions
100 mM dCyd, 100 mM NaNO2, 1 M Acetate buffer
pH 3 7, 37C, 5 min

This intermediate was retained by anion-exchange column
but not by cation-exchange column
NMR and MS supported the assignment.

Overall Reaction Mechanism





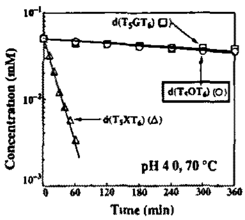
The intermediates are stable.
Halh-life at pH 7 and 37℃
C-diazoate 330 hr (14 days)
G-diazoate: 5 6 min

Basic Properties of dOxo

Sample preparation

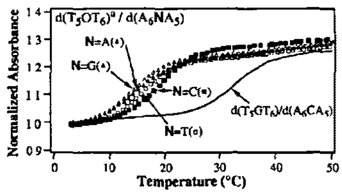
d(T5GT6) ⟶ d(T5OT6) + d(T5XT6) + d(T5GT6)
Separation by HPLC

Stability of N-glycosidic bond



dOxa is as stable as dGuo,
whereas dXao is depurinated readily

Effect of dOxo on duplex stability



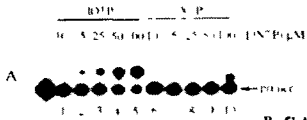
	$d(A_{12})$	$d(A_6GA_5)$	$d(A_6CA_5)$	$d(A_6TA_5)$
$d(T_5OT_6)^a$	15.3°C	14 1°C	19.3°C	16.3°C
$d(T_5GT_6)$	16 8°C	16 9°C	32 8°C	20 4°C
$d(T_5XT_6)^b$	17 3°C	15 6°C	14 8°C	22 3°C

ᵃO oxanosine, ᵇX xanthosine

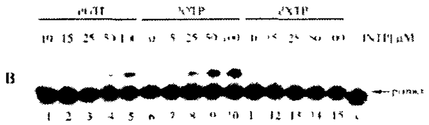
dOxa changes local double helix structure

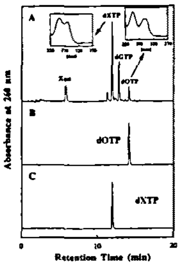
Incorpolation of dOTP and dXTP into Oligonucleotide by Kf

A 5'-ACCCAGTCACGAA
3'-TGGGTCAGTGCTTCGATAGTTGCTAGC



B 5'-ACCCAGTCACGAG
3'-TGGGTCAGTGCTCTGATAGTTGCTAGC



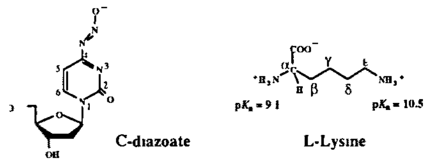


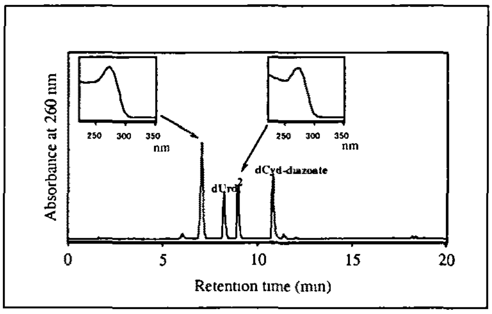
Primer/Template 0 03 µM
Kf 0 015 unit
25°C 5 min

dOTP is incorporated in DNA opposite template C and T.
dXao is incorporated only opposite template C.

International Innovation Center, Kyoto University

Cross-Link: dC-diazoate + L-Lys

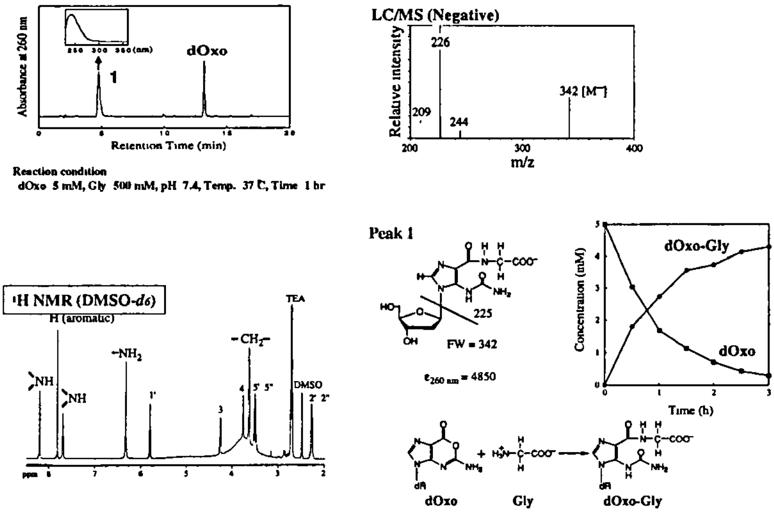




Conditions
C-diazoate 1 mM
L-Lys 10 mM
pH 7 4
temp 37°C
reaction time 3 days (72 h)
* half-life 330 h

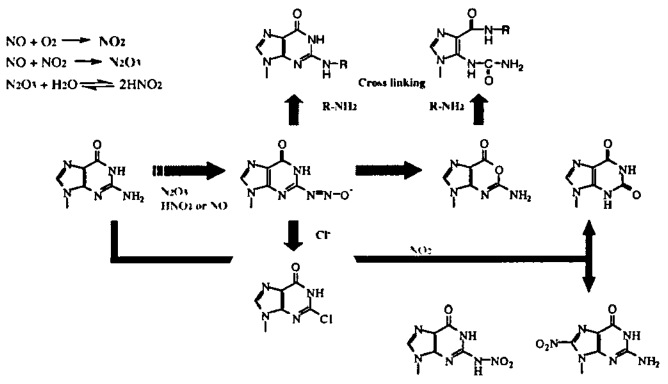
International Innovation Center, Kyoto University

Cross-Link: dOxo + Gly



International Innovation Center, Kyoto University

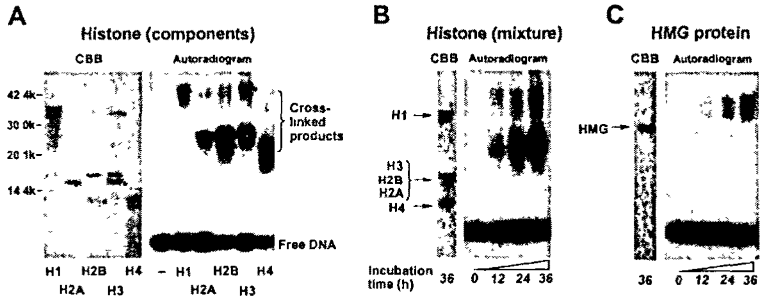
Overall Mechanism for the Reaction of HNO2 (or NO) with dGuo



Conclusion

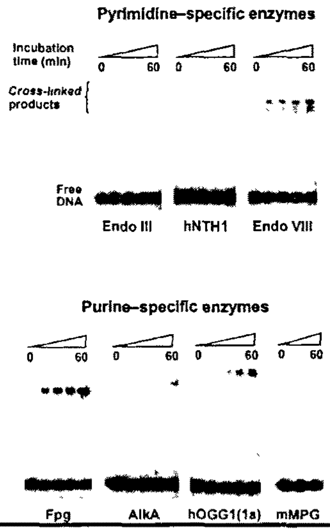
1. Formation of dOxo could cause G/C to A/T conversion.
2. Production of dGuo- and dCyd-diazoates could cause crosslinking between DNA and nuclear compounds which have amino groups such as histone, polyamine et al
3. Above results imply that overproduction of NO may cause DNA damage in cells.

Cross-linking of dOxo-containing Oligo-DNA and Proteins



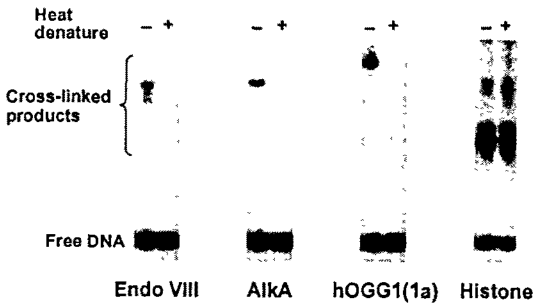
International Innovation Center, Kyoto University

Cross-linking of dOxo-containing Oligo-DNA and DNA Glycosylases



International Innovation Center, Kyoto University

Cross-linking of dOxo-containing Oligo-DNA and Heat-Denatured DNA Glycosylases and Histones



International Innovation Center Kyoto University